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Rozprawa doktorska

Reakcje behawioralne na sytuacje stresowe inwazyjnych, pontokaspijskich gatunków ryb babkowatych i ich rodzimych odpowiedników

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2. **Augustyniak M**, Kobak J, Trojan M, Kakareko T. 2024. Behavioural responses to environmental novelty in demersal, shelter-associated invasive fish and their native analogues. Animal Behaviour, 208:111-126. Impact Factor w roku 2022: 2.5, Q1 w kategorii zoologia

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Spis treści

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Streszczenie

Inwazje biologiczne to proces, w którym organizmy są wprowadzane przy udziale człowieka poza zasięg ich naturalnego występowania, gdzie są w stanie tworzyć stabilne, samoutrzymujące się populacje oraz samodzielnie rozprzestrzeniać się na pobliskie tereny i wywoływać zmiany w zajmowanych ekosystemach. Człowiek intensyfikuje zjawisko inwazji biologicznych w skali przestrzennej i czasowej, znosząc bariery geograficzne i umożliwiając przemieszczanie się organizmów na znaczne odległości w czasie krótszym niż w przypadku naturalnych mechanizmów dyspersji. W konsekwencji gatunki inwazyjne stanowią zagrożenie dla różnorodności biologicznej, a jednym z kluczowych zagadnień w badaniach nad procesem inwazji biologicznych jest poznanie cech gatunków inwazyjnych, w tym behawioralnych, leżących u podstaw ich sukcesu w zajmowaniu nowych terenów.

Nadrzędnym celem rozprawy doktorskiej było poznanie reakcji behawioralnych pontokaspijskich ryb babkowatych (Gobiidae) oraz sympatrycznych gatunków rodzimych na sytuacje stresowe związane z zajmowaniem nowego środowiska. Zagadnienie to jest istotne w kontekście oceny potencjału inwazyjnego specyficznej grupy dennych i terytorialnych ryb, której przedstawicielami są pontokaspijskie Gobiidae. Wykonano trzy eksperymenty, w których testowano dwa gatunki babek rozprzestrzeniających się w wodach słodkich Europy (babka łysa *Babka gymnotrachelus* i babka szczupła *Neogobius fluviatilis*), oraz gatunki rodzime o zbliżonej do nich biologii i ekologii (odpowiednio: głowacz białopłetwy *Cottus gobio* oraz kiełb *Gobio gobio*). W pierwszym eksperymencie badano zachowania obronne ryb w konfrontacji z drapieżnikiem. Drugi eksperyment dotyczył odpowiedzi behawioralnych ryb na nowe, nieznane środowisko (test opuszczania kryjówki, test otwartego pola) oraz nagłą zmianę w znanym środowisku (test reakcji na nowy obiekt). W ostatnim eksperymencie badano reakcje behawioralne ryb na informacje socjalne, tj. generowane zachowaniem innych osobników (zarówno własnego jak i innych gatunków), świadczące o zagrożeniu lub lokalizacji zasobów w nowym środowisku.

Stwierdzono, że badane gatunki inwazyjnych ryb babkowatych: (1) nie wykazywały bardziej skutecznych zachowań obronnych w konfrontacji z drapieżnikiem niż gatunki rodzime; (2) okazały się bardziej odważne, tj. mniej związane z kryjówką i bardziej intensywnie eksplorujące otoczenie w zetknięciu z nowym środowiskiem w porównaniu z rodzimymi gatunkami; (3) były w stanie wykorzystywać

wewnątrz- i międzygatunkowe informacje socjalne o zagrożeniu w zespołach ryb, a babka szczupła – także informacje o lokalizacji zasobów. Rezultaty badań sugerują, że inwazyjne babki mogą mieć konkurencyjną przewagę nad gatunkami rodzimymi w wyniku bardziej intensywnej eksploracji otoczenia i eksploatacji zasobów w nowo zajmowanych środowiskach. Porównywalna z rodzimymi rybami podatność babek na chwytanie przez drapieżniki, wskazuje na potencjał powodowania zmian w sieciach troficznych przez włączanie inwazyjnych Gobiidae do diety rodzimych drapieżników i redukowanie liczebności babek na zajmowanych terenach. Aczkolwiek, przeciwstawną rolę mogą odgrywać tutaj zdolności babek do wykrywania zagrożenia na podstawie zachowań innych osobników i potencjalnego unikania bezpośredniej konfrontacji z drapieżnikiem, sprzyjające ich ekspansji. Wyniki uzyskane w ramach niniejszej pracy doktorskiej dostarczają nowej wiedzy na temat potencjału inwazyjnego pontokaspijskich, bentosowych ryb z rodziny babkowatych oraz podkreślają ważność behawioru jako mechanizmu mogącego wpływać na sukces inwazyjnych zwierząt na nowo zajmowanych terenach.

Abstract

Biological invasion is a process of introducing organisms with human assistance outside their native range, where they can sustain a stable population, spread to nearby areas and cause changes in invaded ecosystems. Human intensifies this process on spatial and temporal scales by removing geographical barriers and allowing organisms to move for longer distances in a shorter time compared to natural dispersal mechanisms. As a consequence, invasive species constitute a serious threat to global biodiversity. One of the key aspects of biological invasion studies is the insight into features of invasive species, including behavioural characteristics, which determine their success in newly settled areas.

The main aim of this dissertation was to expand the knowledge about the behavioural reactions of invasive Ponto-Caspian gobies (Gobiidae) and sympatric native species to stress related to their appearance in the novel environment. Knowing this is important to assess the invasive potential of Ponto-Caspian gobies as a model of a specific group of benthic, solitary fish species. Three experiments were performed on two gobies species spreading in European freshwaters (the racer goby Babka gymnotrachelus and the monkey goby Neogobius fluviatilis) and sympatric native species similar in biology and ecology (the European bullhead Cottus gobio and the gudgeon Gobio gobio, respectively). In the first experiment, antipredator behaviours facing predatory fish were assessed. The aim of the second experiment was to assess behavioural reactions to novel, unfamiliar environments (the shelter occupancy test, the open field test) or a sudden change in a familiar environment (the novel object reaction test). In the last experiment, behavioural reactions to social information, i.e. information provided by the behaviour of others (con- and heterospecifics), related to danger or resource location in novel environments were studied.

It was found that invasive gobies: (1) did not show more effective antipredator behaviours compared to natives; (2) were bolder, i.e. less associated with the shelter and explored the novel, unfamiliar environment more extensively than natives; (3) were able to use social information about danger from con- and heterospecifics, and the monkey goby did the same also for social information about resource location. These results suggest that invasive gobies may gain a competitive advantage over sympatric native species by more extensive exploration and resource exploitation in newly settled environments. The similar susceptibility to predation compared to native prey species pointed out the potential of

invasive gobies to affect food webs by including invasive Gobiidae in the diet of the native predators, thus reducing the abundance of gobies in invaded ecosystems. Although, the ability of gobies to detect danger based on the behaviour of other individuals and potentially avoid direct confrontation with a predator, may favour their expansion. The results obtained under this dissertation provide novel insight into the invasive potential of benthic Ponto-Caspian gobies and underlie the role of behaviour as a potential mechanism affecting invasion success in newly settled areas.

Inwazje biologiczne

Zjawisko inwazji biologicznych

Rozprzestrzenianie się (dyspersja) organizmów, jest naturalnym procesem, leżącym u podstaw kształtowania się różnorodności biologicznej na Ziemi. Proces ten może mieć formę aktywną, polegającą na wykorzystaniu własnych zdolności ruchu lub pasywną, wykorzystującą różne wektory przenoszenia, jak zwierzęta, wiatr czy wodę (Ricciardi 2013, Najberek i in. 2020). Inwazje biologiczne to proces, w którym organizmy są wprowadzane (introdukowane) przy udziale człowieka (celowo lub przypadkowo) poza zasięg ich naturalnego występowania, gdzie są w stanie tworzyć stabilne, samoutrzymujące się populacje oraz samodzielnie rozprzestrzeniać się na pobliskie tereny i wywoływać zmiany w zajmowanych ekosystemach (Richardson i in. 2000, Ricciardi 2013). Istota problemu inwazji biologicznych polega na tym, że działalność człowieka intensyfikuje proces rozprzestrzeniania się organizmów w skali przestrzennej i czasowej. W wyniku globalnej rozbudowy sieci transportowych oraz intensywnej wymiany towarów organizmy są przemieszczane na znaczne odległości (międzykontynentalne) w czasie krótszym niż wynika to z wykorzystania naturalnych mechanizmów dyspersji. Działalność człowieka znosi dotychczasowe bariery geograficzne, stwarzając możliwość dyspersji zwierząt na obszary dotychczas dla nich niedostępne. Może to mieć charakter celowy, kiedy człowiek świadomie przemieszcza organizmy z jednego miejsca do innego, jak np. ryby do celów hodowlanych i/lub hobbystyczych (akwarystyka, wędkarstwo) (Carlton 1992) czy rośliny do celów ozdobnych (Keller i in. 2011). Ponadto, organizmy mogą być przemieszczane w sposób niezamierzony, przypadkowy, kiedy są transportowane bez wiedzy człowieka. Przykładem jest tutaj transport wielu organizmów wodnych wraz z wodami balastowymi statków (Bailey 2015).

Pojawienie się osobników danego gatunku na nowym terenie, znajdującym się poza zasięgiem rodzimego (naturalnego) występowania, wpisuje się w początkową fazę inwazji biologicznej. Cały proces obejmuje kolejno etapy: transportu, introdukcji, zadomowienia i rozprzestrzeniania gatunku. Jeżeli gatunek obcy (przeniesiony poza zasięg swojego naturalnego występowania) na nowym terenie generuje szkody środowiskowe i/lub w gospodarce człowieka, zyskuje miano gatunku inwazyjnego (Blackburn i in. 2011, Chapple i in. 2012, Najberek i Solarz 2016). Gatunek taki stanowi zagrożenie dla różnorodności biologicznej i może powodować dotkliwe konsekwencje ekologiczne, ekonomiczne, społeczne i zdrowotne (Głowaciński i in. 2011). Gatunki inwazyjne wpływają na funkcjonowanie nowo zasiedlanych ekosystemów, powodując m.in. zmiany w cyklach obiegu pierwiastków (Mack i in. 2000), zmiany strukturalne środowiska (Croll i in. 2005, Kornijów i in. 2010), modyfikacje sieci troficznych (Doherty i in. 2016, David i in. 2017, Kornijów i in. 2018), a także krzyżując się (hybrydyzacja) z gatunkami rodzimymi (Simberloff 1996, Biedrzycka i in. 2012), konkurując z nimi o ograniczone zasoby środowiskowe (Vilà i Weiner 2004) czy stając się żywicielami lokalnych i/lub wektorami nowych gatunków pasożytów (Kvach i Ondračková 2020). Grupa najbardziej inwazyjnych gatunków, tj. o szczególnie dużym potencjale w zajmowaniu nowych terenów, jest stosunkowo mało liczna; np. 69% wszystkich introdukcji gatunków inwazyjnych ryb słodkowodnych na świecie jest związanych z jedynie 61 gatunkami (Toussaint i in. 2016). W związku z tym, inwazje biologiczne prowadzą do sukcesywnego zwiększenia podobieństwa taksonomicznego i funkcjonalnego pomiędzy ekosystemami (Villéger i in. 2011, Su i in. 2021). Bardzo często pojawienie się w danym środowisku gatunku inwazyjnego generuje także straty ekonomiczne (Diagne i in. 2020). Mogą one objawiać się zmniejszeniem plonów spowodowanym działalnością inwazyjnych szkodników upraw (Charles i Dukes 2007), niszczeniem infrastruktury przez zarastanie urządzeń hydrotechnicznych (Haubrock i in. 2022) czy zmniejszeniem atrakcyjności turystycznej danego terenu, ograniczającym przychody (Shwiff i in. 2010). Co więcej, gatunki inwazyjne mogą bezpośrednio zagrażać zdrowiu i życiu człowieka, będąc wektorami chorób, czego przykładem mogą być inwazyjne gatunki komarów, przenoszące takie choroby jak żółta febra, denga czy malaria (Juliano i Lounibos 2005).

Z uwagi na dużą wagę i skalę problemu, tematyka inwazji biologicznych zajmuje ważne miejsce w badaniach z zakresu ekologii i ochrony środowiska, w tym zwłaszcza w kontekście zmian globalnych (Huang i in. 2011). Jednym z kluczowych zagadnień jest poznanie mechanizmów inwazji, w tym cech gatunków inwazyjnych leżących u podstaw konkurencyjnej przewagi nad gatunkami rodzimymi i osiąganiu sukcesu w zajmowaniu nowych terenów. Jednym ze słabo poznanych w tym kontekście aspektów, o potencjalnie dużym znaczeniu w przebiegu inwazji u zwierząt, jest behawior.

Behawior jako mechanizm wpływający na sukces inwazji biologicznych

Behawior można zdefiniować jako złożony systemem zachowań zwierzęcia, przejawiających się w działaniu lub zaniechaniu działania w odpowiedzi na sygnały docierające ze środowiska zewnętrznego lub z wnętrza organizmu (Levitis i in. 2009, Kokocińska i Kaleta 2015). Warto nadmienić, że tak rozumiany behawior obejmuje zachowania zarówno osobnika jak i grupy osobników (Levitis i in. 2009). Behawior może być jednym z mechanizmów wpływających na sukces inwazji. Jak opisali Chapple i in. (2012), różne zachowania mogą przynosić korzyści gatunkom inwazyjnym na różnych etapach inwazji. Na przykład na etapie transportu duże znaczenie może mieć wysoki stopień eksploracji środowiska, który może przyczyniać się do łatwiejszego dostania się na wektor transportu (Sih i in 2004). Z kolei w momencie przybycia na nowe miejsca zachowania socjalne, tj. wchodzenie w interakcje z innymi osobnikami przez np. okazywanie agresji, mutualizm, kooperację (Rubenstein i Rubenstein 2013), mogą decydować o tym, czy osobniki pozostają razem na danym terenie (osobniki socjalne, tworzące zorganizowane grupy), czy rozprzestrzeniają się (osobniki asocjalne) i determinować osiągnięcie zagęszczenia odpowiedniego do wytworzenia stabilnej populacji (Cote i in 2010). W fazie tworzenia stabilnej populacji ważne okazują się zachowania związane z opieką nad potomstwem (Chapple i in. 2012), które zwiększają sukces reprodukcyjny przedstawicieli gatunku inwazyjnego. Kolejnym elementem, związanym z przeżywalnością osobników na różnych etapach inwazji, jest obrona przed drapieżnictwem. Behawior może odgrywać kluczową rolę jako mechanizm obronny, warunkując np. skuteczną ucieczkę ofiary przed atakującym drapieżnikiem (Kelley i Magurran 2003, Szulkin i in. 2006, Pietrzak i in. 2017). Zachowania obronne mogą mieć rozmaite formy, jak unikanie przebywania w miejscach o wysokim stopniu zagrożenia, ukrywanie się, zmniejszenie aktywności, aktywną ucieczkę przed polującym drapieżnikiem czy pozostawanie w bezruchu (Lima i Dill 1990, Mikolajewski i Johansson 2004, Pijanowska i in. 2006, Teplitsky i Laurila 2007, Dawidowicz i in. 2013). Efektywne zachowania obronne, zmniejszające ryzyko pochwycenia przez drapieżnika mogą stanowić mechanizmy wpływające na sukces inwazji (Jermacz i in. 2017, Jermacz i Kobak 2018).

Interesującym aspektem w kontekście inwazji biologicznych są zachowania zwierząt związane z odwagą. Zachowania te mają duże znaczenie ewolucyjne i ekologiczne (White i in. 2013), ponieważ są wpisane w podejmowanie przez zwierzęta decyzji ściśle związanych z przeżywalnością, tzn. wyboru pomiędzy schronieniem się przed drapieżnikami, a aktywnym poszukiwaniem zasobów, takich jak pokarm czy partner do rozrodu (Avarguès-Weber i in. 2013). Odwaga definiowana jest jako zdolność organizmu do podejmowania ryzyka (Wilson i in. 1994, Ballew i in. 2017), a jej miarą może być np. reakcja behawioralna na pojawienie się w nowym, nieznanym środowisku (Brown i in. 2007b, Toms i in. 2010, Mustafa i in. 2019). Stawianie czoła nowym i nieprzewidywanym zagrożeniom związanym z nieznanym środowiskiem czy obecnością drapieżnika może wywoływać u zwierząt stres (Perrot-Minnot i in. 2017). Odpowiedź na ten stres może być realizowana na wielu poziomach organizacji organizmu, począwszy od zmian w gospodarce hormonalnej, przez zmiany w funkcjonowaniu układów krwionośnego i oddechowego, skończywszy na odpowiedzi całego organizmu, wyrażonej m.in. przez zmianę zachowania (Schreck i Tort, 2016, Sopinka i in. 2016). W takiej sytuacji zachowania odważne osobnika, manifestujące się wysoką aktywnością oraz intensywną eksploracją środowiska, mogą przekładać się na szybkie lokalizowanie zasobów i w efekcie sprawniejsze ich pozyskiwanie. W przypadku gatunków inwazyjnych odwaga może zatem zwiększać prawdopodobieństwo osiągnięcia sukcesu na nowo zajmowanych terenach. Z drugiej strony, ten sam repertuar zachowań naraża osobniki na niebezpieczeństwo ze strony drapieżników, zmniejszając ich szanse na utworzenie stabilnej populacji. Gatunki introdukowane poza naturalny zasięg występowania spotykają się z wieloma wyzwaniami związanymi z pojawieniem się w nowym dla nich środowisku. To, jak w takich sytuacjach reagują, jak się zachowują, może determinować ich przetrwanie (Gerlai i in. 1990). Badania reakcji behawioralnych różnych grup zwierząt na nowe środowisko, mogą dostarczyć cennych informacji na temat tego w jakim stopniu odwaga wpisana jest w potencjał gatunków inwazyjnych do zasiedlania nowych terenów.

Kolejnym ważnym zagadnieniem w kontekście inwazji biologicznych są zdolności inwazyjnych zwierząt do wykorzystywania informacji socjalnych w zespołach organizmów w nowo zajmowanych środowiskach. Za informację socjalną uznaje się tę dostarczaną za pomocą zachowania innych osobników (Duboscq i in. 2016), a wykorzystanie jej polega na zmianie własnego zachowania w odpowiedzi na zachowanie prezentowane przez inne osobniki (Bonnie i Earley, 2007). Dla organizmów znajdujących się w nieznanym środowisku może ona stanowić cenne źródło informacji o jego zagrożeniach i zasobach. Umiejętność wykorzystania informacji socjalnych ma przewagę nad zdobywaniem informacji metodą prób i błędów, ponieważ nie naraża organizmu na ryzyko związane z przypadkowym spotkaniem z drapieżnikiem podczas eksploracji środowiska (Bonnie i Earley, 2007). Należy mieć na uwadze, że sygnały nie muszą pochodzić od osobników własnego gatunku. Korzystanie z informacji socjalnych pochodzących od osobników innych gatunków może przynosić te same korzyści, a w przypadku gatunków inwazyjnych wręcz zapewniać przewagę konkurencyjną nad gatunkami rodzimymi, jako że stanowią one dodatkowe źródła informacji o nowo zajętym terenie (Damas-Moreira i in. 2018). W momencie zajmowania nowych terenów liczba osobników gatunku inwazyjnego może być niewielka (Terkel 1995, Camacho-Cervantes i in. 2015) w związku z czym umiejętność wykorzystywania informacji socjalnych od osobników gatunków rodzimych przebywających w pobliżu umożliwia obcym przybyszom szybkie dostosowanie się do warunków panujących w danym siedlisku, co ma duże znaczenie dla ich przetrwania (Tuomainen i Candolin 2011) i sukcesu inwazji.

Inwazje biologiczne w środowiskach wodnych

Szczególnie narażone na utratę bioróżnorodności w wyniku pojawienia się gatunków inwazyjnych są środowiska słodkowodne, zajmujące niecały 1 % powierzchni Ziemi, jednak zapewniające siedliska dla około 30 % gatunków zwierząt kręgowych (Dudgeon i in. 2006). Środowiska te znajdują się pod silnym wpływem antropopresji. Regulacja rzek oraz tworzenie sztucznych połączeń (kanałów żeglugi) pomiędzy izolowanymi wcześniej ciekami i akwenami w ramach rozbudowy sieci transportu wodnego stwarza nowe możliwości dyspersji gatunków inwazyjnych (Bij de Vaate i in. 2002). Zabudowa hydrotechniczna, na którą składają się śluzy, jazy czy zapory budowane do celów hydroenergetycznych, a także modyfikacje koryt rzecznych i zlewni prowadzące do zmian w intensywności spływu powierzchniowego powodują zmiany naturalnych warunków środowiska (ukształtowanie i struktura dna, modyfikacja przepływu wody) skutkujące degradacją siedlisk (Dudgeon i in. 2006) i spadkiem dostosowania zajmujących je organizmów rodzimych. To z kolei zmniejsza ich skuteczność w konkurencji z pojawiającymi się gatunkami inwazyjnymi i zwiększa potencjał tych drugich do zajmowania środowisk zmodyfikowanych przez człowieka (Koehn 2004, Johnson i in. 2008, Raab i in. 2018). Co więcej, wysoka zazwyczaj gęstość zaludnienia na terenach przyległych pociąga za sobą wzrost liczby incydentów wprowadzania gatunków inwazyjnych do wód słodkich, w wyniku ucieczek tych organizmów z hodowli komercyjnych czy amatorskich (prowadzonych w celach konsumpcyjnych, ozdobnych), bądź celowego wypuszczania obcych przybyszy (Nunes i in 2015).

Ważnym czynnikiem powodującym wzrost zagrożenia dla różnorodności biologicznej europejskich wód śródlądowych była rozpoczęta w XVII w. budowa szeregu sztucznych kanałów, które finalnie utworzyły trzy drogi wodne umożliwiające ekspansję gatunków inwazyjnych z regionu pontokaspijskiego, określone później mianem korytarzy inwazji: (1) korytarz północny, łączący rzekę Wołgę z Morzem Bałtyckim, (2) korytarz centralny, łączący rzeki Dniepr i Ren oraz (3) korytarz południowy, łączący rzeki Dunaj i Ren (Bij de Vaate, 2002). Przyspieszyło to znacznie zarówno aktywne, jak i pasywne (np. przez przyczepianie się do kadłubów łodzi, transport w wodach balastowych) rozprzestrzenianie się gatunków z tych terenów do Europy Centralnej i Zachodniej (Bij de Vaate 2002; Gallardo i Aldridge 2015). Region pontokaspijski obejmuje Morza Czarne, Kaspijskie i Azowskie oraz dolne odcinki uchodzących rzek wraz z systemem łączących je cieków (Ricciardi, 2001). Burzliwa historia tego regionu, charakteryzująca się wielokrotnymi transgresjami i regresjami wód, ruchami górotwórczymi oraz następującymi po sobie okresami glacjacji i deglacjacji (Dumont, 2000) doprowadziła do wytworzenia się na tych terenach specyficznej, eurytopowej fauny, tolerującej szerokie wahania temperatury czy zasolenia (Rewicz i in. 2014). Kilka gatunków makrobezkręgowców pochodzących z rejonu pontokaspijskiego jest łączonych ze zmianami sieci troficznych na nowo zajmowanych terenach czy wymieraniem gatunków rodzimych (Soto i in 2023). Przykładem są tutaj dwa gatunki: małż racicznica zmienna *Dreissena polymorpha* (Pallas 1971) oraz kiełż *Dikerogammarus villossus* (Sovinsky 1894). Oba gatunki samodzielnie lub wchodząc ze sobą w interakcje stanowią zagrożenie dla rodzimej bioróżnorodności na nowo zajmowanych terenach (Aldridge i in. 2014, Rewicz i in. 2014). Region pontokaspijski ma także swoich przedstawicieli, jeśli chodzi o inwazyjne gatunki kręgowców, jakimi są ryby z rodziny babkowatych (Gobiidae).

Ryby babkowate z regionu pontokaspijskiego to gatunki związane z dnem cieków i zbiorników wodnych, głównie bentosożerne, wykazujące przywiązanie do kryjówek (Grabowska i in. 2023). Ryby te charakteryzują się niewielkimi rozmiarami ciała (do kilkunastu centymetrów długości), krótkimi cyklami życiowymi (4-5 lat), wczesnym osiąganiem dojrzałości płciowej (1-2 rok życia), tarłem porcyjnym (do 3 w ciągu roku), a dodatkowo wykazują opiekę nad potomstwem, co wyróżnia je na tle wielu gatunków rodzimych konkurujących z nimi na nowo zajmowanych terenach (Daoulas i in. 1993, Kottelat i Freyhof 2007, Patzner i in. 2012, Grabowska i Przybylski 2014). Przemieszczają się z wykorzystaniem wspomnianych wcześniej korytarzy inwazji, a dynamika kolonizowania przez nie nowych terenów w wielu systemach rzecznych Europy, np. Dunaju, Renu, Wisły, Odry czy Newy spowodowała, że inwazje ryb babkowatych z regionu pontokaspijskiego uznawane są za jedne z najbardziej spektakularnych w Europie (Copp i in. 2005). Co więcej, wraz z wodami balastowymi statków dostały się one także do Wielkich Jezior w Ameryce Północnej (Kornis i in. 2012). Na nowo zajmowanych terenach ryby babkowate wywołują zmiany w zajmowanych biocenozach.

Konkurują o zasoby stanowiąc zagrożenie a nawet wypierając gatunki rodzime (Kornis i in. 2012, Kakareko i in. 2013, van Kessel i in. 2016). Wpływają na zmiany w sieciach troficznych, w których stają się zarówno nowymi drapieżnikami (Janssen i Jude 2001, Barton i in. 2005) jak i ofiarami (Almqvist i in. 2010, Płąchocki i in. 2012). Wywołują też zmiany w populacjach pasożytów, stając się żywicielami lokalnych i/lub wektorami nowych gatunków (Kvach i Ondračková 2020, Ondračková i in. 2021). Warto wspomnieć, że w ekosystemach słodkowodnych Europy nie występują autochtoniczne gatunki ryb z rodziny babkowatych. Zatem w wodach tych, pontokaspijskie babki wchodzą w interakcje z filogenetycznie odległymi, ale często zbliżonymi pod względem ekologii rodzimymi przedstawicielami ichtiofauny. Z powodu zmian jakie omawiane ryby babkowate mogą powodować w zajmowanych ekosystemach, dogłębne poznanie czynników (cech tych zwierząt oraz powiązanych z nimi mechanizmów) mogących kształtować ich konkurencyjną przewagę nad sympatrycznymi gatunkami rodzimymi, wydaje się zagadnieniem szczególnej wagi w kontekście ochrony lokalnej bioróżnorodności.

Badania wykonane w ramach pracy doktorskiej

Informacje wstępne i cele badań

Istnieją prace dotyczące oceny poziomu odwagi w kontekście zdolności rozprzestrzeniania się inwazyjnych gatunków ryb, lecz dotyczą one przede wszystkim gatunków pelagicznych (np. Rehage i Sih 2004, Ashenden i in. 2017, Lukas i in. 2021), tj. przebywających w toni wodnej (Torres i Bailey 2022). Ponadto, badania te rzadko obejmują porównanie cech behawioralnych pomiędzy inwazyjnymi i sympatrycznymi, tj. występującymi razem w środowisku, gatunkami rodzimymi (Pawelec-Olesińska 2020, Heermann i in. 2022, Sales i in. 2023). Także badania nad wykorzystaniem informacji socjalnych, w głównej mierze prowadzone są na gatunkach pelagicznych (np. Harpaz i Schneidman 2020, Webster i Laland 2012), a prac przedstawiających porównanie inwazyjnych i rodzimych gatunków pod względem zdolności w tym zakresie jest niewiele (np. Camacho-Cervantes i in. 2015). Nikły jest stan wiedzy na temat behawioralnych mechanizmów rozprzestrzeniania się pontokaspijskich Gobiidae, będących doskonałym przykładem typowo bentosowych inwazyjnych ryb słodkowodnych, tj. związanych z dnem cieków i zbiorników wodnych (Torres i Bailey 2022), spędzających większość czasu na dnie, często w kryjówkach (Mills i Mann 1983, Kottelat i Freyhof 2007, Grabowska i in. 2016, Jakubčinová i in. 2017). Z uwagi na przydenny tryb życia, reakcje behawioralne tych ryb na sytuacje stresowe związane z rozprzestrzenianiem się w nowym środowisku, nie powinny być przewidywane w oparciu o wyniki badań prowadzonych na gatunkach typowo pelagicznych. Z racji tego, że są to zwykle gatunki terytorialne, nie formujące ławic, również ich zdolności do korzystania z informacji socjalnych, zwłaszcza pochodzących od innych gatunków, są w porównaniu z rybami pelagicznymi słabo poznane.

Aby uzupełnić luki w wiedzy dotyczące potencjalnego wpływu behawioru na sukces inwazji bentosowych ryb babkowatych z regionu pontokaspijskiego, w ramach niniejszej rozprawy doktorskiej przeprowadzono serię eksperymentów w kontrolowanych warunkach w laboratorium. Aby stwierdzić, w jakim stopniu babki różnią się pod względem wybranych cech behawioralnych od gatunków rodzimych, co jest istotne w kontekście oceny potencjału inwazyjnego babek, testom poddano także gatunki będące rodzimymi odpowiednikami (potencjalnymi konkurentami) obcych przybyszy. Gatunki były porównywane w parach (inwazyjny gatunek i rodzimy odpowiednik), a kryterium doboru stanowiło podobieństwo ekologii i biologii oraz współwystępowanie obu gatunków w środowisku.

Nadrzędnym celem badań było poszerzenie wiedzy na temat specyfiki behawioru inwazyjnych ryb z rodziny babkowatych, w tym zwłaszcza reakcji na sytuacje stresowe, w kontekście potencjału tych ryb do wypierania rodzimych przedstawicieli ichtiofauny i zajmowania nowych terenów. Cele szczegółowe badań dotyczyły porównania dwóch gatunków babek (babki łysej, babki szczupłej) oraz odpowiadających im rodzimych gatunków ryb (głowacz białopłetwy, kiełb) pod kątem reakcji behawioralnych na następujące czynniki środowiskowe:

1. Obecność drapieżnika. Testowano tutaj zachowania obronne (antydrapieżnicze) ryb, wraz z oceną ich skuteczności, w sytuacji bezpośredniej konfrontacji z drapieżnikiem.
2. Nowe środowisko. W tym przypadku testowano reakcje behawioralne ryb na stres wywołany nowym otoczeniem (nieznanym środowiskiem lub nagłą zmianą w znanym środowisku).
3. Informacje socjalne. Testowano zdolności ryb do wykorzystania informacji socjalnych o zagrożeniu (źródło stresu) lub zasobach (źródło pożywienia).

Każdy z wykonanych w ramach pracy doktorskiej trzech eksperymentów, opisanych w dalszej części rozprawy, był zaplanowany i przeprowadzony pod kątem realizacji jednego z wymienionych powyżej zadań badawczych (celów szczegółowych).

Badane ryby

W wodach europejskich obecnych jest 6 inwazyjnych gatunków ryb babkowatych (Copp i in. 2005). Do badań wybrano dwa z nich, rozprzestrzeniające się w wodach słodkich Europy: babkę łysą *Babka gymnotrachelus* (Kessler 1857) oraz babkę szczupłą *Neogobius fluviatilis* (Pallas 1814). Przedstawiciele obu gatunków to organizmy małe, osiągające do kilkunastu centymetrów długości ciała i dożywające wieku 4-5 lat (Kottelat i Freyhof 2007). Spotykane są na podłożach piaszczystych, kamienistych i żwirowych (Płąchocki i in. 2020). Nie posiadają pęcherza pławnego, przez co większość czasu spędzają na dnie cieków i zbiorników wodnych (Teletchea i Beisel 2018). Podczas przemieszczania się wykazują strategię typu burst-and-hold (Egger i in. 2021), przejawiającą się wykonywaniem swoistych skoków w toni wody, a następnie swobodnym opadaniem i zajmowaniem nieruchomej pozycji na dnie aż do wykonania kolejnego skoku (Tierney i in. 2011).

Jak wspomniano wcześniej, aby określić wpływ prezentowanych zachowań na potencjał inwazyjny badanych gatunków babek porównano ich zachowanie z behawiorem sympatrycznych gatunków rodzimych. Babkę łysą zestawiono z rodzimym głowaczem białopłetwym *Cottus gobio* (Linneusz 1758). Głowacz białopłetwy, przedstawiciel rodziny głowaczowatych (Cottidae), jest szczególnie narażony na interakcje z inwazyjną babką łysą ze względu na wysokie podobieństwa w ekologii i biologii, obejmujące np. przywiązanie do kryjówek, brak pęcherza pławnego oraz strategię poruszania się typu burst-and-hold (Mills i Mann 1983, Tomlinson i Perrow 2003, Grabowska i Grabowski 2005, Kornis i in. 2012). Oba gatunki współwystępują w środowisku (Jażdżewski i in. 2012, Kakareko i in. 2016, Janáč i in. 2018), a badania laboratoryjne wykazały wyższość babki łysej nad głowaczem białopłetwym w konkurencji o pokarm (Kakareko i in. 2013) i kryjówkę (Jermacz i in. 2015, Błońska i in. 2016, Grabowska i in, 2016). Babkę szczupłą natomiast zestawiono z kiełbiem *Gobio gobio* (Linneusz 1758). Pomimo tego, że w odróżnieniu od babki szczupłej, kiełb jest gatunkiem przebywającym w toni wodnej i zaliczanym do tworzących ławice (Stott 1967, Egger i in. 2021), oba gatunki wykazują podobne preferencje siedliskowe i współwystępują ze sobą na podłożu piaszczystym (Kottelat i Freyhof 2007, Płąchocki i in. 2020). Obserwacje terenowe wykazały spadek zagęszczenia kiełbia w odpowiedzi na wzrost zagęszczenia babki szczupłej (Jakovlić i in. 2015), co może świadczyć o występowaniu negatywnego wpływu gatunku inwazyjnego na rodzimy w środowiskach, w których współwystępują.

W badaniach wykorzystano zarówno gatunki inwazyjne (b. łysa i b. szczupła) jak i gatunek objęty w Polsce częściową ochroną gatunkową (głowacz białopłetwy) na mocy Rozporządzenia Ministra Środowiska z dnia 16 grudnia 2016 r w sprawie ochrony gatunkowej zwierząt. Przed rozpoczęciem badań uzyskano od Regionalnego Dyrektora Ochrony Środowiska w Bydgoszczy wymagane zgody dotyczące chwytania, transportu i przetrzymywania ww. osobników w związku z realizacją badań naukowych (zgody nr: WOP.6401.4.5.2017.MO, WOP.6401.4.19.2018.MO, WOP.6401.4.52.2022.MO). Uzyskano także zgody od Lokalnej Komisji Etycznej w Bydgoszczy na wykorzystanie zwierząt w procedurach wykonanych w ramach każdego z eksperymentów (zgody nr: 50/2017, 51/2021, 53/2022).

Eksperymenty

Zasadniczy element prezentowanej rozprawy doktorskiej stanowią trzy eksperymenty behawioralne, w których testowano reakcje ryb na: (1) obecność polującego drapieżnika, (2) stres wywołany przez nowe środowisko oraz (3) zachowanie innych osobników (wykorzystanie informacji socjalnych). Opublikowane prace oparte na tych eksperymentach oraz manuskrypt przygotowany do wysłania do czasopisma stanowią podstawę niniejszej rozprawy.

W pierwszym eksperymencie, związanym z pierwszym celem pracy doktorskiej i przedstawionym w publikacji 1 (Augustyniak i in. 2023), badano reakcje obronne ryb oraz efektywność unikania bezpośredniego zagrożenia ze strony drapieżnika. Zasadniczym celem badań było określenie różnic w tym względzie pomiędzy inwazyjnymi i rodzimymi rybami, tj. babką łysą i głowaczem białopłetwym oraz babką szczupłą i kiełbiem. W zajmowanych środowiskach inwazyjne gatunki ryb babkowatych modyfikują istniejące zależności troficzne często stając się ważnym komponentem diety rodzimych drapieżników (Reyjol i in. 2010, Płąchocki i in. 2012). W związku z wysokim zagęszczeniem inwazyjnych babek na zajmowanych przez nie terenach (Kakareko i in. 2009, 2016) wysoki udział tych ofiar w diecie rodzimych drapieżników do tej pory wiązano z wysoką dostępnością w środowisku (Płąchocki i in. 2012, Crane i Einhouse 2016, Mikl i in. 2017). Nie można jednak wykluczyć, że wykazują one mniej efektywne zachowania obronne w porównaniu do gatunków rodzimych. Sugeruje to praca Kłosińskiego i in. (2022), gdzie w kontakcie z substancją alarmową kiełb wykazał bardziej złożone zachowania obronne niż babka szczupła. W ramach omawianego eksperymentu badano zachowania ryb w kluczowym okresie najbardziej intensywnych interakcji pomiędzy drapieżnikiem i ofiarą, tj. po ich wzajemnym wykryciu w środowisku, do momentu pochwycenia i połknięcia ofiary przez drapieżnika. Umożliwienie bezpośredniego kontaktu ofiary z drapieżnikiem było konieczne w celu uzyskania pełnego obrazu efektywności mechanizmów obronnych testowanych ofiar, w tym także tych umożliwiających uwolnienie się i ucieczkę ofiary po schwytaniu przez drapieżnika. W eksperymencie testowano interakcje pojedynczych osobników ofiar eksponowanych na oddziaływanie okonia (*Perca fluviatilis*), drapieżnika występującego pospolicie na terenach zajmowanych przez wszystkie badane gatunki ofiar i żerującego na tych gatunkach (Nesbø i in. 1999, Płąchocki i in. 2012, Kakareko i in. 2016). Zachowanie drapieżnika także podlegało ocenie, co ułatwiło interpretację uzyskanych wyników. **Postawiono dwie hipotezy: (1) gatunki inwazyjne wykazują inne zachowania antydrapieżnicze w sytuacji bezpośredniego zagrożenia ze strony drapieżnika niż gatunki rodzime; (2) zachowania antydrapieżnicze inwazyjnych gatunków są mniej efektywne i w rezultacie gatunki te są łatwiejszymi do upolowania ofiarami niż gatunki rodzime.**

Zadaniem drugiego eksperymentu, przedstawionego w publikacji 2 (Augustyniak i in. 2024), była realizacja drugiego z postawionych w ramach pracy doktorskiej celów przez poznanie reakcji behawioralnych badanych ryb na stres wywołany nagłym zetknięciem z nieznanym środowiskiem. Aby zwiększyć prawdopodobieństwo prawidłowej interpretacji zachowań ryb, wykonano serię różnych testów, a wyniki każdego z nich interpretowano w oparciu o wyniki pozostałych. Wykonano trzy kluczowe, najczęściej stosowane w tego rodzaju badaniach u zwierząt (Wright i in. 2006, Brown i in. 2007a, Collier i in. 2017) testy odwagi: (1) test opuszczania kryjówki; (2) test reakcji na nowy obiekt oraz (3) test otwartego pola. Każdy z tych testów odzwierciedla wyzwania napotykane przez gatunki inwazyjne w procesie inwazji, a także przez gatunki rodzime w wyniku antropogenicznego przekształcania środowiska. W teście opuszczania kryjówki badany osobnik musi dokonać wyboru pomiędzy bezpiecznym pozostaniem wewnątrz, a opuszczeniem kryjówki i eksploracją nieznanego środowiska, co może go narazić na niebezpieczeństwo spotkania z drapieżnikiem. W przypadku gatunków inwazyjnych, odwaga manifestująca się mniejszym przywiązaniem do kryjówek w porównaniu z sympatrycznymi gatunkami rodzimymi, może zapewniać przewagę w postaci bardziej intensywnej eksploatacji zasobów środowiska. Może być to związane z częstszym żerowaniem czy przemieszczaniem się na większe odległości w poszukiwaniu pokarmu. Test reakcji na nowy obiekt pozwala ujawnić odpowiedź behawioralną osobnika na zmiany, w tym pojawienie się nieznanego obiektu (np. wprowadzanych przez człowieka elementów konstrukcyjnych zabudowy hydrotechnicznej) w znanym dla niego środowisku. Szybsze zbliżenie się do takiego obiektu i jego inspekcja (zebranie informacji) pozwala szybciej określić ewentualne korzyści lub zagrożenia związane z obiektem i w rezultacie szybciej dostosować się do zaistniałej zmiany. Wykazujące większą odwagę gatunki inwazyjne mogą zyskiwać tutaj przewagę nad gatunkami rodzimymi, albowiem szybsza ocena sytuacji może przekładać się na wzrost efektywności pozyskiwania zasobów lub unikania zagrożenia, zwiększając szanse na przeżycie. Test otwartego pola może z kolei ujawnić reakcje behawioralne badanych osobników na nieznane środowisko pozbawione kryjówek. W przypadku inwazyjnych gatunków ryb może to być związane z możliwościami kolonizowania przez nie cieków strukturalnie przekształconych przez człowieka w wyniku prac regulacyjnych czy zabudowy hydrotechnicznej, o ujednoliconej budowie dna (np. kanały, zbiorniki zaporowe). Bardziej odważne osobniki gatunków inwazyjnych w porównaniu z rodzimymi, intensywniej eksplorujące takie środowiska, mogą zyskiwać przewagę z powodu większych zdolności dyspersji, zajmowania stref otwartego dna lub pokonywania barier w postaci takich siedlisk. **W przypadku tego eksperymentu jako hipotezę przyjęto, że gatunki inwazyjne są bardziej odważne, co przejawia się krótszym przebywaniem w kryjówce, szybszą inspekcją nowego obiektu, dłuższym przebywaniem w otwartym polu, a także intensywniejszą jego eksploracją, w porównaniu z gatunkami rodzimymi**.

W trzecim eksperymencie wykonanym w ramach prezentowanej pracy doktorskiej, nawiązującym do ostatniego z postawionych celów i przedstawionym w manuskrypcie, testowano zdolności wykorzystania informacji socjalnych u badanych ryb. Zastosowano tutaj zestaw eksperymentalny, w którym źródłem informacji socjalnych były filmy z zarejestrowanym odpowiednim zachowaniem ryb - dawców sygnałów (demonstratorów). Testowano dwa rodzaje sygnałów: (1) alarmowy, pochodzący od osobników wystraszonych, oraz (2) pokarmowy, pochodzący od osobników żerujących. W pierwszym przypadku, sygnał traktowano jako silniejszy, albowiem w naturze błędne odczytanie takich informacji i brak odpowiedniej reakcji behawioralnej może wiązać się z bezpośrednim i natychmiastowym zagrożeniem życia osobnika obserwującego (odbiorcy informacji). Zastosowanie tego sygnału powinno wywołać u badanych gatunków stres, a odpowiedzią powinna być prezentacja zachowań obronnych. Był to więc zasadniczy czynnik nawiązujący do bodźców prezentowanych w pozostałych eksperymentach (publikacje 1. i 2.). W drugim przypadku, błędne odczytanie i reakcja na sygnały socjalne w środowisku naturalnym może wiązać się z pośrednim zagrożeniem życia, w dłuższej perspektywie czasowej, poprzez zmniejszenie efektywności zdobywania pokarmu. Pomimo tego, że w założeniu ten bodziec nie wywoływał stresu u testowanych osobników, włączenie go do badań pozwoliło na bardziej dogłębne poznanie i zrozumienie roli sygnałów socjalnych w ekologii badanych gatunków. Zbieranie informacji o środowisku z wykorzystaniem doświadczenia innych osobników przez obserwację ich zachowań ma tę przewagę nad zdobywaniem ich samodzielnie przez aktywną eksplorację, że pozwala osobnikowi obserwującemu zaoszczędzić energię oraz zredukować ryzyko przypadkowego spotkania z drapieżnikiem (Bonnie i Earley 2007). Może to być użyteczne w nowym środowisku, gdzie szybkość zdobycia informacji o zagrożeniach oraz dostępnych zasobach może determinować przetrwanie osobnika (Terkel 1995). Dodatkowym atutem może być zdolność do wykorzystania tego typu informacji od przedstawicieli innych gatunków (Damas-Moreira i in. 2018), co wydaje się wyjątkowo korzystne w przypadku osobników inwazyjnych, często obecnych w małych zagęszczeniach na nowym terenie w początkowym etapie inwazji (Terkel 1995, Camacho-Cervantes i in. 2015). Zatem, jeśli gatunki inwazyjne są w stanie wykorzystywać takie informacje bardziej efektywnie od gatunków rodzimych, tzn. odczytywać więcej rodzajów sygnałów zarówno od przedstawicieli swojego jak i innych gatunków, zwiększają swoje szanse przeżycia i potencjał konkurencyjny wobec gatunków rodzimych na nowo zajmowanych terenach. **Postawiona hipoteza zakładała, że gatunki inwazyjne w porównaniu do rodzimych mają większe zdolności wykorzystywania informacji socjalnych i reagują na wszystkie rodzaje sygnałów użytych w eksperymentach, tj. silniejszy (alarmowy) i słabszy (pokarmowy) zarówno od przedstawicieli własnego jak i obcego gatunku. Natomiast gatunki rodzime reagują na silny sygnał niezależnie od gatunku demonstratorów, jednak nie są w stanie wykorzystywać przypuszczalnie najsłabszego sygnału, tj. od żerujących osobników obcego gatunku.**

Wyniki i ich omówienie

Badania wykonane w ramach pierwszej części pracy doktorskiej (publikacja 1 – Augustyniak i in. 2023), poświęcone ocenie reakcji behawioralnych ryb w sytuacji bezpośredniego zagrożenia ze strony polującego drapieżnika, w dużym stopniu potwierdziły postawione hipotezy. Wykazano, że inwazyjne babki mają odmienne wzorce zachowań obronnych i są dla drapieżnika podobnie lub mniej trudnymi do upolowania (chwytania i połknięcia) ofiarami, niż rodzime gatunki. W przypadku pierwszej pary testowanych gatunków, drapieżnik szybciej chwytał i połykał osobniki inwazyjnej babki łysej w porównaniu z osobnikami rodzimego głowacza białopłetwego. Głowacz w konfrontacji z drapieżnikiem był ogólnie mniej aktywny, ale w trakcie ucieczki wykazywał bardziej złożone reakcje behawioralne niż babka łysa, polegające na częstych zmianach kierunku ruchu, prawdopodobnie dezorientując drapieżnika. Co istotne, po schwytaniu głowacz białopłetwy w odróżnieniu od babki był w stanie niekiedy wyswobodzić się z jamy gębowej drapieżnika i uciec. W przypadku drugiej pary, składającej się z babki szczupłej i kiełbia, ponownie gatunek rodzimy prezentował szerszy repertuar zachowań antydrapieżniczych w porównaniu z gatunkiem inwazyjnym. Osobniki kiełbia znacznie częściej podejmowały ucieczki przed schwytaniem w porównaniu z osobnikami babki szczupłej, jednak nie wpływało to na czas polowania, mierzony od rozpoczęcia testu do połknięcia ofiary przez drapieżnika. Mniej efektywne od gatunków rodzimych mechanizmy obronne mogą wyjaśniać duży udział inwazyjnych babek w diecie rodzimych drapieżników i wskazywać na potencjał babek do modyfikowania sieci troficznych na nowo zajmowanych terenach.

Badania przeprowadzone w ramach drugiej części doktoratu (publikacja 2 – Augustyniak i in. 2024), dotyczące reakcji behawioralnych badanych gatunków związanych z nieznanym środowiskiem, potwierdziły założoną przez nas hipotezę. W konfrontacji z nowym środowiskiem oba gatunki inwazyjne wykazały się większą odwagą i mniejszym przywiązaniem do kryjówek w porównaniu do rodzimych gatunków. Zarówno babka łysa jak i babka szczupła eksplorowały środowisko poza kryjówką w większym stopniu niż ich rodzime odpowiedniki. Wszystkie badane gatunki zbliżały się do nowego obiektu po jego wprowadzeniu do środowiska, jednak gatunki rodzime łączyły to zachowanie ze zmniejszoną aktywnością, co może sugerować próbę wykorzystania nowego obiektu jako potencjalnego schronienia. Inwazyjne gatunki babek w tym samym czasie wykazywały większą niż u gatunków rodzimych aktywność w strefach niezwiązanych z nowym obiektem, interpretowaną jako aktywną eksplorację środowiska. Odpowiedzi behawioralne na znalezienie się w otwartym polu prezentowane przez gatunki rodzime były bardziej złożone niż te prezentowane przez gatunki inwazyjne. Jednak interpretując wyniki testu otwartego pola w świetle wyników pozostałych testów stwierdzono, że oba gatunki inwazyjne były bardziej odważne i eksplorowały arenę w większym stopniu niż ich rodzime odpowiedniki. Mniejszy związek inwazyjnych gatunków ryb babkowatych z kryjówkami oraz większa eksploracja nowych środowisk w porównaniu do sympatrycznych gatunków rodzimych może sprzyjać dalszej ekspansji tych gatunków inwazyjnych szczególnie w siedliskach ze znacznym udziałem strefy otwartego dna, gdzie potencjalna konkurencja siedliskowa z sympatrycznymi gatunkami rodzimymi może być mniejsza niż w siedliskach bogatych w kryjówki.

Wyniki badań zrealizowanych w ramach trzeciej części omawianej pracy doktorskiej (manuskrypt), tj. eksperymentu dotyczącego wykorzystania informacji socjalnych, częściowo wspierają hipotezę, że inwazyjne babki przewyższają pod tym względem gatunki rodzime. W przypadku bodźca alarmowego, pochodzącego od wystraszonych osobników innego gatunku, oba gatunki inwazyjne zareagowały ograniczeniem aktywności, a babka szczupła dodatkowo unikała strefy położonej bezpośrednio przy demonstratorach wykazujących reakcję alarmową. Oba te zachowania stanowią jeden z podstawowych mechanizmów obronnych (Lima i Dill 1990, Teplitsky i Laurila 2007) co pokazuje, że gatunki inwazyjne rozpoznały sygnał socjalny oraz zinterpretowały go prawidłowo, jako świadczący o zagrożeniu. Spośród gatunków rodzimych również kiełb wykazywał behawioralną reakcję obronną w odpowiedzi na ten rodzaj sygnału niezależnie od rodzaju demonstratorów. Natomiast głowacz białopłetwy przejawiał mniejsze zdolności w tym względzie od pozostałych gatunków, reagując tylko na sygnał pochodzący od osobników własnego gatunku. W przypadku sygnałów socjalnych pochodzących od żerujących osobników, wydaje się, że oba gatunki rodzime oraz babka szczupła przejawiały zdolność szacowania nie tylko potencjalnych zysków związanych z żerowiskiem, ale także strat związanych z nasiloną konkurencją o pokarm po dołączeniu do żerujących demonstratorów. Ryby te wykazywały większe zainteresowanie sygnałem pochodzącym od demonstratorów będących potencjalnie słabszymi konkurentami. Jedynie babka łysa wydawała się nie odczytywać w ten sposób sygnałów pochodzących od żerujących osobników innego gatunku. Babka łysa wykazywała większe zainteresowanie demonstratorami własnego gatunku, narażając się na potencjalnie silną konkurencję wewnątrzgatunkową. Wykonane badania wskazują, że zdolności inwazyjnych babek do odczytywania szerszego spektrum sygnałów socjalnych świadczących o zagrożeniu, tj. sygnałów zarówno wewnątrz-jak i międzygatunkowych, w porównaniu z rodzimymi rybami, mogą prowadzić do bardziej efektywnego unikania drapieżnictwa przez niektóre babki na nowo zajmowanych terenach, a w konsekwencji – zwiększenia przeżywalności osobników i sukcesu inwazji. Ponadto, zaobserwowane unikanie żerujących ryb babkowatych przez rodzime ryby, może sprzyjać ustępowaniu tych drugich z optymalnych żerowisk, a ułatwiać zajmowanie nowych terenów przez te pierwsze.

Podsumowanie i wnioski

Najważniejsze wnioski z badań przeprowadzonych przeze mnie w ramach pracy doktorskiej na dwóch inwazyjnych, pontokaspijskich gatunkach ryb z rodziny babkowatych (babka łysa *Neogobius gymnotrachelus,* babka szczupła *Neogobius fluviatilis*) oraz odpowiadających im rodzimych gatunkach (głowacz białopłetwy *Cottus gobio*, kiełb *Gobio gobio*) są następujące:

1. Inwazyjne gatunki babek nie wykazują bardziej skutecznych zachowań obronnych w porównaniu do sympatrycznych gatunków rodzimych w konfrontacji z drapieżnikiem.
2. Inwazyjne babki wykazują bardziej odważne zachowania (mniejsza zależność od kryjówek, bardziej intensywna eksploracja środowiska) w odpowiedzi na nagłe pojawienie się w nieznanym środowisku lub nagłe zmiany znanego środowiska w porównaniu do gatunków rodzimych.
3. Inwazyjne babki są w stanie wykorzystywać informacje socjalne o zagrożeniu od otaczających je osobników gatunków rodzimych. Częściowo są także w stanie wykorzystać informacje socjalne świadczące o lokalizacji pokarmu

Rezultaty badań sugerują, że behawior może odgrywać istotną rolę w kształtowaniu potencjału inwazyjnego pontokaspijskich ryb babkowatych. Dzięki bardziej odważnym zachowaniom inwazyjne gatunki ryb babkowatych mogą zyskiwać przewagę nad gatunkami rodzimymi w wyniku bardziej intensywnej eksploracji otoczenia i eksploatacji zasobów w nowo zajmowanych środowiskach. Babki okazały się względnie łatwymi do upolowania ofiarami dla drapieżników, co wskazuje na potencjał tych organizmów do powodowania zmian w sieciach troficznych. Aczkolwiek, inwazyjne babki mogą, przynajmniej częściowo, kompensować brak bardziej skutecznych zachowań antydrapieżniczych w porównaniu do gatunków sympatrycznych większymi zdolnościami wykrywania obecności drapieżnika, na podstawie zachowań innych osobników rożnych gatunków obecnych w środowisku, co może pozwalać na uniknięcie bezpośredniej konfrontacji z drapieżnikiem.

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Differences in predator-avoidance behavior between two invasive gobies and their native competitors

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Abstract

Globally, fish are frequently introduced beyond their native range. Some, like Ponto-Caspian gobies, are becoming invasive, achieving high colonization rates and constituting frequent prey for native predators. However, little is known about the effectiveness of antipredator behaviors of the invaders, which may shape their role in the invaded community and contribute to the invasion success. We compared antipredator behaviors of invasive gobies and native fish species after their detection by the predator, when the danger becomes direct. We studied 2 fish pairs, each consisting of an invasive and native species co-occurring in the environment and belonging to the same prey guild: (1) the racer goby *Babka gymnotrachelus* versus European bullhead *Cottus gobio*, (2) the monkey goby *Neogobius fluviatilis* versus gudgeon *Gobio gobio*, facing a naïve predator (the Eurasian perch *Perca fluviatilis*). We analyzed behaviors of single prey individuals (escaping, staying in shelter, and activity) and single predators (activity, searching, following, capturing, and latency to prey consumption). In the predator presence, the bullhead was less active and more often managed to escape after capture than the racer goby. The gudgeon escaped before the capture more often than the monkey goby. The predator succeeded later with the bullhead compared to racer goby, whereas no differences in ingestion time occurred between the gudgeon and monkey goby. The results suggest that, in terms of hunting effort of native predators, the invasive gobies are equivalent to or more profitable prey than their native analogs, which can facilitate the integration of the gobies into local food webs.

Key words: antipredator behavior, fish behavior, invasive prey, native predator, predator–prey interactions.

Invasive species are one of the greatest threats to biodiversity and community structure (Rodriguez 2006; Hughes et al. 2020; Dueñas et al. 2021). They spread spectacularly and have a strong impact on the environment (Ricciardi 2013). One important effect here is that the invasive species create new trophic relationships, and modify existing ones in recipient ecosystems. They can affect native species directly, by predation and competition (Rodriguez 2006; Levine 2008; Błońska, Grabowska, et al. 2016; Haubrock et al. 2020), or indirectly, for example, by altering predator–prey relationships of natives and therefore modifying the structure of food webs (David et al. 2017; Haubrock et al. 2019). This includes cases where invasive species influence native predators as their new prey (Crane et al. 2016; Stellati et al. 2019). Such influence can have various forms (Venable et al. 2019), depending on how effective the new prey is in predator avoidance compared to the native prey, and how these prey species interact with each other. Invasive prey can have a detrimental effect on native prey species through apparent competition (Holt 1977) due to increased predation pressure (Noonburg and Byers 2005; Castorani and Hovel 2015). On the other hand, different trophic scenarios are possible between 2 prey species that share a common predator (Harmon and Andow 2004). Positive indirect effects of one prey (here: invasive) on the other (native) can occur when an increase in the density of the former impairs the predator's functional response to the latter due to predator saturation or predator switching (Abrams and Matsuda 1996; Webster and Almany 2002). Finally, invasive prey could constitute an integral part of the local food web, for example, by modifying the trophic level in the way that it consists almost entirely of invasives, which makes even native predators highly dependent on those invasive prey species (Bissattini et al. 2021). There is a need for understanding how particular biological features of invasive species determine their availability as prey for native predators, and how they perform compared to native species belonging to the same prey guild, that is, in situations when their distributions overlap temporally and spatially and they share the same predators. Assessing prey antipredatory strategies in this context is important in a broader perspective for predicting the outcome of new trophic linkages created by invasive species, and their impact on food webs.

Prey exhibit predator-induced defenses involving changes in morphology (e.g., McCollum and Leimberger 1997; Boersma et al. 1998; Dahl and Peckarsky 2002), life history (Tams et al. 2018), and behavior (Lima and Dill 1990; Sparrevik and Leonardsson 1999; Johansson et al. 2004). Behavioral defenses involve spatial avoidance, increased hiding,

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decreased activity, diet change (Mikolajewski and Johansson 2004; Teplitsky and Laurila 2007), escape, deterrence, and freezing (Lima and Dill 1990). These responses are considered components of the predator-avoidance strategy in prey, representing a sequence of events taking place consecutively from the detection of the predator by the prey until it is finally consumed or successfully escapes (Kelley and Magurran 2003). Particularly noteworthy is the final period of a predator–prey interaction, when a prey individual is detected by a predator and prey responses are most intense. This is the peak active phase of predator avoidance that precedes the time when prey is eaten (ingested). The moment of ingestion is decisive and should be taken into account, as capture does not necessarily mean death. Indeed, prey can defend themselves, for example, by using spines and/or toxins (Hasegawa et al. 2021) and successfully avoid ingestion even after capture.

On the global scale, fishes are one of the taxa most commonly introduced outside their native range (Gozlan 2008; Haubrock et al. 2022). We focused on the gobies, as their invasions are considered amongst the most impressive freshwater fish invasions within Central and Western Europe (Copp et al. 2005; Roche et al. 2013). Six invasive goby species are currently present in European waters (Copp et al. 2005), including the racer goby *Babka gymnotrachelus* (Kessler 1857) and the monkey goby *Neogobius fluviatilis* (Pallas, 1814). These species have spread successfully in freshwaters, especially in the river Vistula (Płąchocki et al. 2020), which is one of the largest rivers in the Baltic Sea region (HELCOM 2018). We investigated the antipredator behavior of the racer and monkey gobies by pairing them with native species from the same guild, as this may help to answer the question of whether this aspect of their biology can constitute an advantage to the invasive gobies compared to the local species threatened by their invasions. The racer goby is often found in the same locations as the European bullhead *Cottus gobio* (Linnaeus, 1758) (Janáč et al. 2018). Substantial habitat overlap between these 2 species was revealed in a lowland European river in locations with medium water velocities on stony and gravely substrate (Kakareko et al. 2016). The racer goby was able to outcompete the European bullhead for food (Kakareko et al. 2013) and shelter (Jermacz et al. 2015; Błońska, Kobak, et al. 2016; Grabowska et al. 2016) in laboratory experiments. On the other hand, the monkey goby occupies sandy bottom areas, which are also optimal for the gudgeon *Gobio gobio* (Linnaeus, 1758) (Kottelat and Freyhof 2007; Płąchocki et al. 2020). Increasing monkey goby densities have been observed to coincide with declines in gudgeon populations (Jakovlić et al. 2015).

The invasive gobies are a common, often dominant dietary item of predators in invaded areas (Reyjol et al. 2010; Płąchocki et al. 2012), which may suggest the lower security of the invader in the mixed-species guild. However, it is important to note that high densities and thus high availability of the gobies as prey for predators are also important. According to the theory of optimal foraging (Werner and Hall 1974; Pyke and Starr 2021), predators are expected to select the types of prey that provide the greatest net energy gain, and these are usually the most abundant and easily captured organisms available in the environment. High abundances of the gobies are recorded in colonized environments (Kakareko et al. 2009, 2016), and so they are potentially widely available prey for predators. Nevertheless, in an experimental study on behavioral reactions to predation cues (prey skin extracts), the gudgeon exhibited thigmotaxis and reduction in horizontal and vertical mobility, while the monkey goby did not show any of those behaviors (Kłosiński et al. 2022). This suggests that weaker antipredator responses of invasive gobies may indeed contribute to their susceptibility to predation in invaded areas. In our current research, we assessed whether the behavioral responses of the gobies to direct predation danger follow the same pattern, that is, are less pronounced in invasive than native species. This, in the light of studies indicating that the invasive gobies are a common, often dominant dietary item of predators in invaded areas (Reyjol et al. 2010; Płąchocki et al. 2012) may suggest the lower security of the invader in the mixed-species guild.

Our main goal was to assess the differences in antipredatory behavior and its effectiveness between 2 invasive Ponto-Caspian goby fish (Gobiidae) and their native counterparts. We focused on prey behavior in the final, the most active stage of a predator–prey interaction, that is, when a reciprocal detection by both sides (predator and prey) has occurred, until a successful ingestion of prey or avoidance of predation. We hypothesized that (1) under direct predation danger, the invasive gobies would present qualitatively different behavior than their native counterparts, displaying different sets of species- or family-specific traits. This is because the invasives, although their habitat requirements are similar to those exhibited by their native counterparts, belong to a taxonomically different, specific family of fish: freshwater Gobiidae (in our research, natives were from Cottidae and Cyprynidae families) that are among the most invasive species in Europe (Copp et al. 2005). (2) Antipredator behavior of the invasive gobies would be less pronounced and effective, that is, would make them easier to be caught and ingested by the predator compared to the native prey species. This is based on the assumption that the invaders in a novel range can benefit from the allocation of more energy resources to growth and reproduction at the cost of weaker antipredatory defenses, which makes them better competitors than the native species in the same area (in accordance with the Evolution of Increased Competitive Ability hypothesis, Blossey and Notzold 1995; Callaway and Ridenour 2004).

We compared results only within the above-mentioned pairs, as they were composed of species coexisting and interacting with each other in the same environments. We assumed that to become effective invaders, alien organisms need to perform better than the natives encountered in a particular co-occupied habitat, rather than generally in all communities.

Materials and Methods

The main idea of the experiment

We focused on checking if the behavioral differences between particular prey species make them more or less difficult to capture and, most importantly, successfully ingest during a direct predator encounter, that is, in the phase when the prey has been detected and exposed to the predator attack. Therefore, the approach enabling the physical contact of the prey with the predator was crucial for answering the questions we posed. We focused on particular prey characteristics which make them easier or more difficult to catch (i.e., behavior). As this is difficult to observe in a natural, heterogeneous environment, we needed to use laboratory experiments to separate the features of interest from the influence of environmental conditions (bottom substrate, macrophytes, water flow, etc.). Thus, we designed an experimental setup to enable the predator to hunt directly on the prey of a given species in an identical, standardized environment to check the influence of species-specific traits (mobility, morphology) in defense against predator attacks. The prey had the opportunity to hide inside a shelter (mesh area accessible to prey but not to the predator) or swim freely outside, facing the predator (Figure 1). To make sure that both prey species in each pair will have the same opportunities and conditions at the start of the experiment, we chose naïve, laboratory-reared specimens of the Eurasian perch *Perca fluviatilis* (Linnaeus, 1758) as predators. The perch is often found in habitats occupied by all the tested prey species (invasive and native) (Nesbø et al. 1999) and foraging on them (Płąchocki et al. 2012; Kakareko et al. 2016). As the Ponto-Caspian gobies are mostly eaten by medium-sized predators (Reyjol et al. 2010; Płąchocki et al. 2012), the perch represents an optimal model species to study predator effects on the invasive gobiids and co-occurring native fish species. The use of naïve perch allowed us to eliminate the potential perch preference for one of the studied species resulting from its experience acquired in the natural environment. As we had a limited number of perch individuals, we decided to expose both prey species in the pair to the same perch individual (i.e., each perch individual had the opportunity to interact with both prey species within a given pair, one after the other). However, it must be acknowledged that the predator might gain some experience after consuming the first prey individual. To control for this, we applied a 2 × 2 cross-over design, with various predator individuals offered either a goby or a native species as the first prey.

Animals

We collected the prey fish from the wild in July 2019 and kept them in stock tanks for at least 1 month before the start of experiments. European bullhead and racer goby were collected from the river Brda in central Poland (53°08′52.5″N 17°58′10.5″E) by a diver using an aquarium net. Gudgeon and monkey goby were collected using electrofishing (EFGI 650, Bretschneider Spezial Elektronik, Germany) from the river Pilica in east central Poland (51°45′50.1″N 21°08′55.5″E). We used different fishing methods due to the characteristics of the species and environments. The European bullhead and racer goby in the river Brda were mainly located in shelters (under rocks, roots, etc.) that were easier to be accessed by a diver than by electrofishing. The gudgeon and monkey goby were located on the open bottom, where the electrofishing method was effective. The differences in methods did not disturb the results, as fish were compared in pairs collected from the same environment, using the same method. All the fish were of 0+ age, without any external signs of sexual maturity and thus we did not determine their sex.

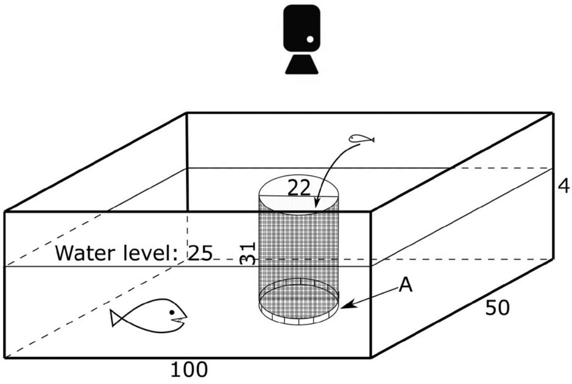

Figure 1. Experimental setup. The predator (perch) was placed in an experimental tank alone to acclimatize. A single prey individual was placed always inside a mesh cylinder acting as a hideout for prey (A). During a single trial, a perch individual was confronted with both prey species from the pair (one after another) in a sequence varying among replicates (native before invasive or invasive before native). Dimensions are given in centimeters.

After capture, we transported the fish in plastic bags containing water and oxygen to the air-conditioned laboratory and held them in 350-L stock tanks (20–30 individuals per tank) filled with conditioned tap water (temperature maintained by air conditioning at 16.1 ± 0.5 °C, pH 8.15 ± 0.15, electrical conductivity 608.4 ± 4.5 µS/cm, oxygen level 8.13 ± 0.25 mg/L and 82.5 ± 3.06%; measured with Multi 340i Meter, WTW, Weilheim, Germany) and equipped with standard aquarium filters and aerators. The photoperiod was set at a 14:10 h light:dark cycle with lights on at 0700 h. The stock tanks were equipped with ceramic and stony shelters and had no bottom substrate. We fed the fish daily ad libitum with frozen chironomid larvae and exchanged water in the tanks once a week (ca. 30% of the water volume) to ensure appropriate level of animal welfare.

Naïve Eurasian perch *P. fluviatilis* was bred from larvae obtained during controlled reproduction of wild breeders captured during commercial catches in early April in accordance with the previously established procedure (Żarski et al. 2011). Fertilized eggs were incubated in a flow-through recirculating system. Larvae hatched on day 8th post-fertilization and were reared at 14 °C, photoperiod 16:8 h light:dark, and oxygen concentration of 8.5 mg/L. Larvae were fed ad libitum with mixed *Artemia* sp. nauplii (INVE, Belgium) and a commercial formulated diet (Perla Larva Proactive 5.0, TrouvitNutreco, The Netherlands) 6 times a day. After 30 days of rearing, juveniles of perch were fed with a commercial diet (Perla Larva Proactive 4.0) and frozen Chironomidae larvae. At the age of about 2 years, the perch (40 specimens) was transported from the breeding facility to the air-conditioned laboratory and kept in an 800-L stock tank filled with conditioned tap water. For 3 months before the start of the experiments, the perch was kept in our laboratory in the same light and temperature conditions as the prey species.

The fish were weighed in a bucket with water before the start of the experiment, and their total body length was measured with ImageJ 1.49v program (freeware by W.S. Rasband, U.S. National Institutes of Health, Bethesda, MD, USA) using digital images taken from the recorded videos.

Although in our research we exposed the tested prey individuals to direct physical contact with a predator, this was the only way to obtain answers to the questions raised. Nevertheless, we did our best to adhere to the ASAB/ABS (2019) guidelines for the use of animals in research by providing them with appropriate housing conditions and obtaining permission from the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (statement no. 50/2017 from 28 September 2017). The housing conditions guaranteed animal welfare, which was manifested by the overall activity and food intake of the fish throughout the research period. We did not notice any external signs of stress or disease (e.g., unnatural body shape, skin changes, swimming problems). After the experiments, the European bullhead and gudgeon that remained uneaten or were not used in the experiments were released where they were caught. Other fish (invasive gobies and Eurasian perch) were euthanized by an overdose of Tricaine Methanesulfonate (MS-222) and disposed of.

Experimental setup

Experiments were conducted in a 200-L tank (100 cm × 50 cm × 40 cm, length × width × height) filled with conditioned tap water and isolated from external stimuli with Styrofoam screens. In the center of the tank, we placed a cylinder (height: 31 cm, diameter: 22 cm) made from a wireframe and plastic mesh (1 mm in diameter) (Figure 1). There was a 1.5-cm gap between the tank bottom and the lower edge of the mesh (marked as A in Figure 1). Thus, prey fish could use it as a shelter, while a predator was too large to get inside. Above the tank, we placed an IP video camera (Samsung SNB-6004P, Changwon, South Korea), which could catch the view of the entire experimental tank. Before the experiments, the tank was equipped with a filter and air stone to maintain appropriate water quality. The filter and air stone were pulled out after a prey fish was placed in the experimental tank to prevent water surface movement, which could disturb the video analysis. The photoperiod and water temperature in the experimental tank were the same as in the stock tanks (14:10 h light:dark cycle, c.a. 16 °C).

Experimental procedure

During each trial, a single predator (perch specimen) was confronted with a single prey individual in 2 successive rounds, so that each predator interacted with both prey species from the pair, one after another. The experiment started by placing a single predator in the experimental tank at 2100 h (Figure 2). After 12 h, we fed it ad libitum with Chironomidae larvae to standardize its hunger level. The experiment was continued only when the predator consumed food, indicating its acclimation to the experimental setup. Twenty-four hours after predator feeding (i.e., at 0900 h), we removed the filter and air stone and placed the first prey specimen from a particular pair of prey species in the experimental tank inside the shelter (mesh cylinder) (first round). Then, the predator had 8 h to consume (i.e., swallow) the prey. The timing of prey ingestion was recognizable based on the movements of the perch's operculum. The capture of the prey was followed by intense movements of the gill lids. The cessation of these movements was considered as the swallowing of the prey, because it never happened that the prey was released from the mouth of the predator after this event. If the prey individual was not consumed, we removed it from the experimental tank. Whether or not the prey was eaten, 24 h after the first prey specimen was placed in the experimental tank, we fed the predator again with Chironomidae larvae ad libitum. The filter and air stone were placed back into the tank. After the following 24 h, we removed the filter and air stone again and placed the second prey specimen (at 0900 h), belonging to the other prey species of the given pair (second round). For each trial, we selected prey individuals of similar sizes (in total length) in each pair. The predator had another 8 h to consume the prey and that was the end of a single trial (Figure 2). We exchanged about 25–30% of water volume between trials. The interval between trials was 28 h. Within each prey species pair, the predators were divided into 2 groups, one facing first the invasive prey and then the native one, and the other confronted consecutively with the native prey and then the invasive one. Each predator individual was used in only 1 trial with 2 rounds (prey individuals).

Each prey species pair was studied separately. In total, we included 11 trials for the European bullhead (mean ± *SD*: individual weight = 0.53 ± 0.16 g; length = 4.51 ± 0.57 cm)/racer goby (0.50 ± 0.28 g; 4.39 ± 1.07 cm) and 15 trials for the gudgeon (0.87 ± 0.28 g; 5.86 ± 1.07 cm)/monkey goby (0.88 ± 0.30 g; 5.36 ± 1.18 cm) pairs in the analysis. There were no differences in weight (paired *t*-test for the European bullhead/racer goby: $t_{10} = -0.70$, $P = 0.494$; for the gudgeon/monkey goby: $t_{13} = -0.26$, $P = 0.801$) and length ($t_9 = 0.40$, $P = 0.703$ and $t_9 = 1.16$, $P = 0.279$, respectively) between prey individuals in each pair. The weight and length of the perch specimens used for the European bullhead/racer goby pair were 43.6 ± 17.4 g and 19.86 ± 3.34 cm, whereas the weight and length of the perch exposed to the gudgeon/monkey goby pair were 46.1 ± 17.2 g and 21.73 ± 2.76 cm, respectively. There were no differences in perch weight and length between the 2 prey pairs (*t*-test: $t_{22} = 0.34$, $P = 0.735$ and $t_{18} = -1.36$, $P = 0.189$, respectively).

Video analysis

Each round of the trial lasted 8 h or until the prey was consumed by the predator. The videos were analyzed semi-automatically using the BORIS 7.9.7 software (Friard and Gamba 2016; Behavioral Observation Research Interactive Software, freeware, www.boris.unito.it). We noted several continuous events (long-term episodes, for which the duration was determined and expressed as % of the total experiment time, i.e., 8 h or till the prey ingestion, if not stated otherwise below) and point events (short-term incidents, for which the number of occurrences were determined) concerning prey and predator behaviors, based on Savino and Stein (1989) and Beauchamp et al. (2007). All noted variables are included in Table 1.

All the videos were analyzed by the same person to avoid any differences due to the subjective assessment by the observer.

Statistical analysis

We performed a Principal Component Analysis (PCA) on the correlation matrix, separately for prey and predator behaviors in each prey pair, to reduce the number of behavioral variables and detect possible relationships between them. The principal components were extracted based on their eigenvalues greater than 1. When explaining the meaning of the obtained principal components, we took into account the original variables with absolute values of their loadings higher than 0.5 after Kaiser-Varimax rotation. The principal components determined by the PCA were analyzed using a 2-way mixed analysis of variance (ANOVA) for cross-over designs (separate for

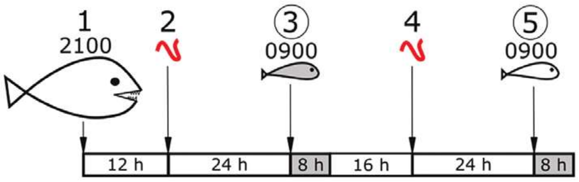

Figure 2. Experimental procedure. 1—releasing the predator (perch) to the tank; 2—the first feeding of the predator with Chironomidae larvae ad libitum; 3—placing the first prey individual in the tank; 4—the second feeding of the predator with Chironomidae larvae ad libitum; 5—placing the second prey individual (of different species than in step 3) in the tank. White rectangles indicate predator acclimation periods. Numbers in circles and gray rectangles indicate the recorded and analyzed periods (rounds) of the trial.

Table 1. The list of variables noted during the video analyses

Prey		Predator	
Behavior	**Description**	**Behavior**	**Description**
Continuous events			
Inactivity	The prey individual not swimming in any part of the tank	Inactivity	The predator not swimming
Exploration	The prey individual actively exploring the tank, undisturbed by the predator	Search	The predator swimming with no obvious signs of interest in the prey individual
Staying in shelter	The prey individual staying inside the cylinder, inaccessible to the predator	Latency to consume the prey	The time from the prey introduction to its successful consumption by the predator (continuous, measured in s) – the prey that survived the experiment was assigned the maximum value of 8 h (28 800 s)
Point events			
Escape	The prey individual rapidly moving away from the predator present in a close distance	Following	The predator maneuvering toward the prey individual to maintain a close distance and seek an opportunity to attack
		Strike	The predator attempting to grasp, injure, or stun prey
		Capture	The predator catching the prey, prey disappearing in the predator’s mouth

each prey species pair). Factors in the ANOVA were set as (1) round of the experiment (the first and second prey individuals of 2 different species, offered consecutively to the predator) as a within-subject factor, (2) sequence (the sequence of offering the prey species to the predator: invasive after native or native after invasive) as a between-subject factor. The effect of prey species was coded in this design indirectly as a round × sequence interaction, whereas the factor sequence indicated potential carryover effects (Díaz-Uriarte 2002; Jones and Kenward 2003). The data were mostly normally distributed (Shapiro–Wilk test) and variances were homogenous (Levene test). All analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp.).

Results

The original behavioral variables included in the analysis are presented in Supplementary Materials A and B. The PCA (Table 2) extracted 2 principal components for prey behavior and 3 principal components for predator behavior in the European bullhead versus racer goby pair, as well as 2 principal components for prey behavior and 2 principal components for predator behavior in the gudgeon versus monkey goby pair.

European bullhead versus racer goby

All individuals of the European bullhead and racer goby were ingested by the predator. The principal component meanings were assigned as follows: (1) prey behaviors: Activity (PC1), Escape (PC2); (2) predator behaviors: Activity (PC1), Efficiency of attacks (PC2), Delay in success (PC3). The prey species differed from each other (as indicated by a significant round × sequence interaction) in the activity (Table 3A): European bullhead was less active than racer goby (Figure 3A). Both prey species showed similar escape responses (Table 3B; Figure 3B). There was a significant effect of the round on predator activity (Table 3C): The perch were less active during round 2 (data not shown) regardless of the prey sequence. The efficiency of perch attacks was similar when facing both prey species (Table 3D; Figure 3D). However, the predator succeeded later when foraging on the European bullhead than on the racer goby (Table 3E; Figure 3E).

Gudgeon versus monkey goby

There were 3 gudgeon and 5 monkey goby individuals which survived the experiment. The following meanings were attributed to the principal components: (1) prey behaviors: Activity (PC1), Escape (PC2); (2) predator behaviors: Predation intensity (PC1), Activity (PC2). There were no differences between the prey species in activity (Table 4A; Figure 4A). However, the prey species differed from each other (as indicated by a significant round × sequence interaction) in their escape behavior (Table 4B; Figure 4B): Gudgeon initiated escape behavior more often than monkey goby (Figure 4D). Additionally, visual inspection of the video recordings revealed that the gudgeon exhibited more sophisticated escape events, performing series of escapes (multiple movements) rather than single point escapes shown by its invasive counterpart. Moreover, prey escape behavior depended significantly on the main effect of round: Both species escaped more often in the second round of the experiment, regardless of the prey sequence (data not shown). The predator behavior was not affected by round, sequence, and prey species (Table 4C, D; Figure 4C, D).

Table 2. Results of the principal component analyses on predator and prey behavioral variables

	PC[a]	λ[b]	% Variance[c]	Variable loadings[d]
European bullhead vs. racer goby				
	Prey behavior			
	PC1 Activity	2.3	57.2	Inactivity (−0,949), Staying in shelter (−0.661), Exploration (0.954)
	PC2 Escape	1.1	25.8	Escape (0.934)
	Predator behavior			
	PC1 Activity	2.1	35.7	Search (0.992), Inactivity (−0.990)
	PC2 Efficiency of attacks	1.4	24.7	Strike (−0.937), Capture (−0.710)
	PC3 Delay in success	1.3	19.6	Latency to consume the prey (0.719), Capture (0.582), Following (−0.635)
Gudgeon vs. monkey goby				
	Prey behavior			
	PC1 Activity	2.0	50.5	Exploration (0.993), Inactivity (−0.991)
	PC2 Escape	1.0	21.1	Escape (0.908)
	Predator behavior			
	PC1 Predation intensity	2.5	41.3	Latency to consume the prey (−0.768), Strike (0.805), Following (0.672), Capture (0.780)
	PC2 Activity	1.9	31.9	Inactivity (−0.989), Search (0.983)

[a] Principal components discriminated by the PCA.
[b] Eigenvalue of the principal component.
[c] Percentage of variance explained by the principal component.
[d] Correlations of measured variables with the principal component (loadings with absolute values higher than 0.5 are shown).

Discussion

We studied the behavior of individual fish exposed to imminent danger from a predator - a naïve Eurasian perch specimen. The prey were invasive Ponto-Caspian gobies and their native counterparts, compared in 2 pairs of species (invasive vs. native) co-occurring in the environment: the racer goby versus European bullhead, the monkey goby versus gudgeon. Our main aim was to determine the differences in antipredator behavior and its effectiveness between the invasive goby fish and their local native analogs. We confirmed our first hypothesis showing that invasive gobies, when facing a direct predator danger, present different behaviors than their native counterparts. However, our second hypothesis was only partially confirmed: In one of the prey species pairs, the native European bullhead turned out to be more difficult for the predator to hunt. On the other hand, in the other species pair, predator efficiency was similar in the presence of both prey species, despite differences in their behavior.

In the first pair of prey species tested, the European bullhead was less active and spent more time in the shelter. The shelter plays a significant role in the biology of both prey species as they spent there most of their time during the day (Mills and Mann 1983; Grabowska et al. 2016, 2019). However, here we have found for the first time that the racer goby used the hideout to a lesser extent than the bullhead facing the direct threat from a predator. The Eurasian perch is a visually oriented predator (Diehl 1988) and more mobile gobies were more visible, thus increasing their risk of being eaten under daylight and clear water conditions in our experiments. Thus, the longer exploration time exhibited by the racer goby suggests that, despite a direct predator danger, the invasive species take more risk and explores the environment. On the other hand, individuals showing greater exploratory activity can more efficiently compete for environmental resources and benefit from improved feeding opportunities, thus showing increased growth and/or fecundity (Huntingford et al. 1990; Fraser et al. 2001). These considerations involve differences on an interspecific level; however, individuals from the same species may also display different personalities, that is, individual differences in boldness, exploration, aggressiveness, etc. (Sih et al. 2004; Kaiser and Müller 2021). Such intraspecific variation can strongly influence a biological invasion (Juette et al. 2014). For example, the presence of bold individuals may help invasive populations to spread further (Chapple et al. 2012). It is possible that, due to its invasive character, the population of the racer goby studied in our experiment contains a higher frequency of bold individuals than that of the European bullhead, which can explain the observed differences. Nevertheless, confirmation of this possibility requires further investigation.

In the second pair of coexisting prey species, the gudgeon exhibited more sophisticated escape events following predator attacks than the monkey goby, performing a series of multiple escapes rather than single movements exhibited by its invasive counterpart. Different escaping strategies may be

Table 3. Two-way ANOVA for cross-over designs to test the behaviors of the European bullhead and racer goby (A, B) as well as their predator (perch) (C–E)

Principal component	Effect	*df*	MS	*F*	*P*
(A) Prey PC1, Activity	Round (R)	1	0.76	1.63	0.233
	Prey species (R × S)	1	10.12	21.64	<0.001*
	Error	9	0.47		
	Sequence (S)	1	1.29	2.16	0.175
	Error	9	0.60		
(B) Prey PC2, Escape	Round (R)	1	0.37	0.45	0.519
	Prey species (R × S)	1	0.04	0.05	0.827
	Error	9	1.09		
	Sequence (S)	1	0.35	0.41	0.540
	Error	9	0.85		
(C) Predator PC1, Activity	Round (R)	1	2.77	7.45	0.023*
	Prey species (R × S)	1	<0.01	<0.01	0.991
	Error	9	0.37		
	Sequence (S)	1	1.43	0.97	0.350
	Error	9	1.47		
(D) Predator PC2, Efficiency of attacks	Round (R)	1	2.29	2.10	0.181
	Prey species (R × S)	1	1.71	1.57	0.243
	Error	9	1.09		
	Sequence (S)	1	0.35	0.41	0.540
	Error	9	0.85		
(E) Predator PC3, Delay in success	Round (R)	1	1.37	4.75	0.057
	Prey species (R × S)	1	3.52	12.16	0.007*
	Error	9	0.29		
	Sequence (S)	1	0.26	0.20	0.666
	Error	9	1.28		

The round of the experiment (the first and second prey individuals of 2 different species offered consecutively to the predator) was set as a within-subject factor, sequence (the sequence of offering the prey species to the predator: invasive after native, or native after invasive, indicating potential carryover effects) as a between-subject factor. The effect of prey species was coded as a round × sequence interaction. Asterisks indicate significant effects at $P < 0.05$.

associated with differences in anatomy and lifestyle characteristics of the prey species, which translate into their swimming abilities. Although the species we selected for both pairs are similar in terms of habitat requirements, body size and shape, they do show anatomical differences affecting their locomotor abilities. The gudgeon has a swim bladder, which makes its lifestyle more benthopelagic (Egger et al. 2021). On the contrary, a swim bladder is absent in the monkey goby (Neilson and Stepien 2011; Teletchea and Beisel 2018) and its pelvic fins form a suction organ increasing its ability to attach to the bottom (Kottelat and Freyhof 2007), which makes it more dependent on the bottom substrate. Thus, gobies are considered poor swimmers (Teletchea and Beisel 2018). Egger et. al. (2021) showed that the gudgeon had better swimming performance compared to another Ponto-Caspian gobiid, the round goby *Neogobius melanostomus* (Pallas, 1814). The authors pointed out that because of the characteristic body shape, which is not adapted to prolonged swimming, benthic fish, such as gobies, display a burst-and-hold swimming mode. In conjunction with the results of Kłosiński et al. (2022), showing the thigmotaxis and dispersion of the gudgeon in response to the alarm substance, this increased number of escapes suggests avoidance of the dangerous area as the main antipredator behavior of this species. Instead, the monkey goby seems to rely on activity reduction allowing it to avoid detection by predators (Čápová et al. 2008; Jakubčinová et al. 2017).

The European bullhead turned out to be more difficult for the predator to hunt than the racer goby, while in the second prey pair predator efficiency was similar in the presence of the gudgeon and the monkey goby. The perch captured the European bullhead more often and needed more time for the final successful ingestion of the bullhead, although spent less time following the bullhead than the racer goby. The higher number of captures of the European bullhead means that this prey species was able to get released from the predator's mouth more often than the racer goby. The bullhead has morphological structures missing in the racer goby, which can be considered as antipredator adaptations reducing capture success: a strong rear-pointing spine protruding from the operculum (Witkowski and Terlecki 2000; Tomlinson and Perrow 2003) and tiny spines on the body, especially near the pectoral fins (Witkowski and Terlecki 2000). The shorter following time may be due to the lower activity of the European bullhead, giving the perch fewer opportunities to actively follow this prey species. All in all, the perch succeeded later when facing the native European bullhead than the invasive racer goby. The strategy that enables the European bullhead to escape from the predator's mouth after capture may be effective in natural, large-scale environments,

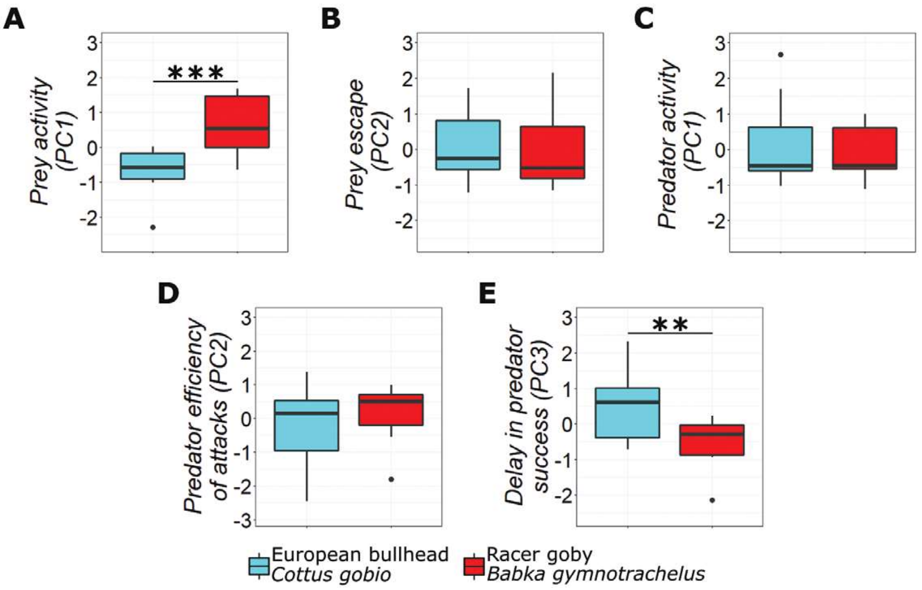

Figure 3. Behaviors of the European bullhead (blue/light) and racer goby (red/dark) (A, B) as well as of their predator (perch) (C–E). A—prey activity; B—prey escape; C—predator activity; D—predator efficiency of attacks; E—delay in predator success. Asterisks indicate significant differences: **P* < 0.05, ***P* < 0.01, ****P* <0.001 (see online for color figures).

Table 4. Two-way ANOVA for cross-over designs to test the behaviors of the gudgeon and monkey goby (A, B), as well as their predator (perch) (C–D)

Principal component	Effect	*df*	MS	*F*	*P*
(A) Prey PC1, Activity	Round (R)	1	0.27	0.25	0.627
	Prey species (R × S)	1	0.02	0.02	0.894
	Error	13	1.08		
	Sequence (S)	1	0.59	0.54	0.474
	Error	13	1.09		
(B) Prey PC2, Escape	Round (R)	1	4.39	6.21	0.027*
	Prey species (R × S)	1	3.67	5.19	0.040*
	Error	13	0.71		
	Sequence (S)	1	2.00	2.83	0.117
	Error	13	0.71		
(C) Predator PC1, Predation intensity	Round (R)	1	<0.01	<0.01	0.998
	Prey species (R × S)	1	0.68	1.28	0.279
	Error	13	0.53		
	Sequence (S)	1	1.21	0.78	0.393
	Error	13	1.68		
(D) Predator PC2, Activity	Round (R)	1	0.18	0.35	0.564
	Prey species (R × S)	1	0.12	0.24	0.631
	Error	13	0.50		
	Sequence (S)	1	0.56	0.34	0.572
	Error	13	1.55		

The round of the experiment (the first and second prey individuals of 2 different species, offered consecutively to the predator) was set as a within-subject factor, sequence (the sequence of offering the prey species to the predator: invasive after native, or native after invasive, indicating potential carryover effects) as a between-subject factor. The effect of prey species was coded as a round × sequence interaction. Asterisks indicate significant effects at $P < 0.05$.

because the predator, discouraged by a failed attack, may lose interest in that particular prey individual. A disincentive here, according to the optimal foraging theory (Pyke and Starr 2021), would be the higher energy costs incurred by the predator due to extended handling time. However, we must be aware that any capture is usually associated with harm to the prey's body. The ability of the bullhead to actively escape from the predator's mouth allows it to survive a direct predator attack, but it is difficult to predict the long-term survival costs of such an escape and this may require further research. We observed that the European bullhead was the only prey studied which exhibited a zig-zagging escape trajectory

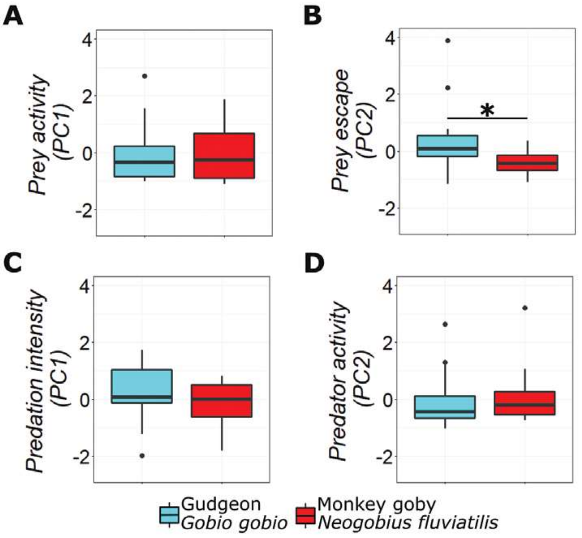

Figure 4. Behaviors of the gudgeon (blue/light) and monkey goby (red/dark) (A, B), as well as of their predator (perch) (C, D). A—prey activity; B—prey escape; C—predation intensity; D—predator activity. Asterisks indicate significant differences: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (see online for color figures).

(personal qualitative observations, not analyzed formally) which is considered an escape behavior with multiple direction changes increasing the chances of survival (Ros et al. 2019). This may also increase the survival of the European bullhead in the wild by confusing the predator, which may lose interest and switch to another prey individual. Our findings suggest that the racer goby, being easier to catch and swallow for the Eurasian perch than the European bullhead, is more beneficial for predators in terms of hunting effort than the native prey from the same guild. On the other hand, the monkey goby, being similarly susceptible to capture and ingestion by Eurasian perch as the gudgeon, is equivalent to its native analog from the same guild as prey for local predators, in terms of the hunting effort of predators.

It should be noted that in our study, antipredatory responses of the gudgeon, although more sophisticated, were not more effective than those of its invasive goby counterpart. Moreover, the European bullhead was finally hunted successfully by the predator, even though it took more time than in the case of the invasive goby. However, we must be aware that our study was geared specifically toward the behavior of prey facing direct threat from a predator, that is, in the phase when the prey has been detected and is exposed to the predator attack. Laboratory experiments were the only possible way to observe the locomotion of fish in repeatable conditions. The strategies of the natives, compared to the invasive gobies, involved a greater number of more varied movements and therefore might be more effective on a wider spatial scale. It is known that the spatial structure of the environment may affect the predator–prey relationships (Mercado-Vásquez and Boyer 2018), because an animal in a confined space cannot perform a straight long-distance relocation, moving away from a dangerous location (Cuddington and Yodzis 2002). In an environment where the space is not limited, the European bullhead may discourage the predator by escaping from its mouth and confuse it by zig-zagging, thus gaining an advantage over the racer goby, whereas the gudgeon would be likely to gain an advantage over monkey goby by moving away from the predator to a safe distance. Nevertheless, laboratory experiments can provide valuable data on interspecific differences in prey behavior, when their results are interpreted taking the above-mentioned limitations into account. The above considerations provide a rationale for believing that under natural conditions, the higher profitability of the invasive gobies as prey for local predators over their native counterparts can be even greater than our laboratory study suggests.

Finally, our results support the idea that the 2 invasive gobies are potentially attractive prey for predators in their novel environments, as we found no greater defensive capacity in these fish compared to the native species. However, extrapolation of these conclusions to other Ponto-Caspian gobies must be done with care. A similar experimental study conducted on the invasive Ponto-Caspian round goby *N. melanostomus* showed that the native predators, the burbot (*Lota lota*) and smallmouth bass *Micropterus dolomieu*, hunted the round goby less efficiently than a native cottid prey species, the mottled sculpin *Cottus bairdii* (Michels et al. 2021). One of the reasons for the discrepancy between the studies may be morphological differences between the gobiids (Jakubčinová et al. 2017). The round goby is discriminated from the other goby species by a significantly deeper caudal peduncle, which may be associated with their better locomotion abilities (Jakubčinová et al. 2017) and, consequently, better ability to escape from attacks of predators. Nevertheless, the shorter, less pronounced, and less diverse defensive behavior we recorded in the invasive gobies is consistent with the study by Kłosiński et al. (2022), who showed that the monkey goby is generally less responsive to the damage-released chemical alarm cues compared to the gudgeon. Thus, our findings suggest that the significant share of the invasive gobies in the predator diet may be not only due to their high density (Płąchocki et al. 2012; Crane and Einhouse 2016; Mikl et al. 2017), according to the optimal foraging theory (Werner and Hall 1974; Pyke and Starr 2021), but also because of the weak behavioral defenses of invasive gobies against predators. Additionally, if this lower defense activity is associated with a reduction in energy expenditure, it may give the gobies an advantage over native fish species in the environments where the predation risk is low, as they may allocate more energy to growth or fecundity rather than to defense against a predator. However, as the invasive gobies do not exhibit more effective defense behavior when facing direct predator danger, they might lose their advantage over native fish in high-risk areas. The above considerations suggest that the invasive gobies, as newly emerged and easily accessible prey, can influence trophic relationships in invaded ecosystems. However, the long-lasting effects of the gobies on populations of native predators and prey are more complex, difficult to predict, and this issue requires further research.

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Conflict of interest statement

The authors declare no conflict of interest

Author contributions

MA: Conceptualization, Resources, Formal analysis, Data interpretation, Visualization, Writing-Original draft preparation. KK: Investigation. JK: Conceptualization, Methodology, Investigation, Formal analysis, Writing-Original draft preparation. PH: Resources, Writing—Review & Editing. PK: Conceptualization, Writing—Review & Editing. MPK: Resources, Investigation, Writing—Review & Editing. ŁJ: Resources, Writing—Review & Editing. TK: Conceptualization, Methodology, Investigation, Writing-Original draft preparation Supervision, Funding acquisition.

Data availability

The data that support the findings of this study are available from the corresponding author, MA, upon request.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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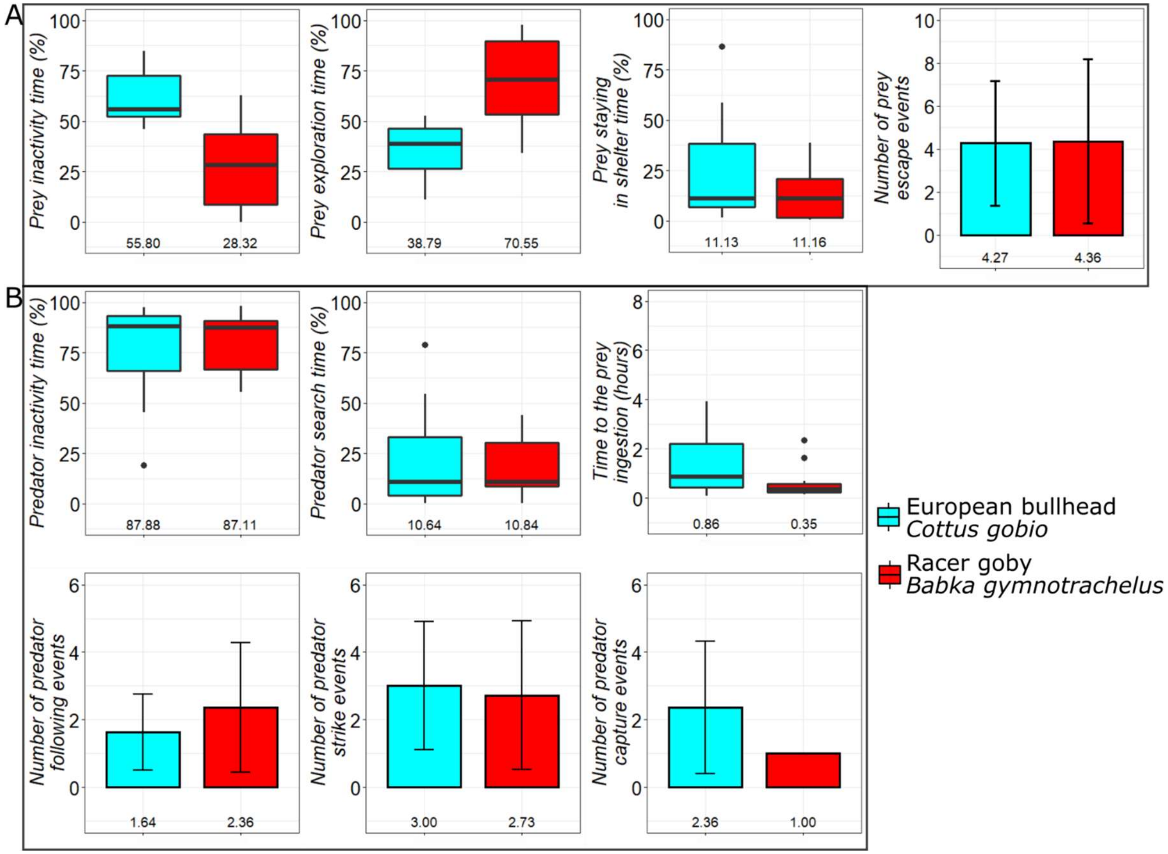
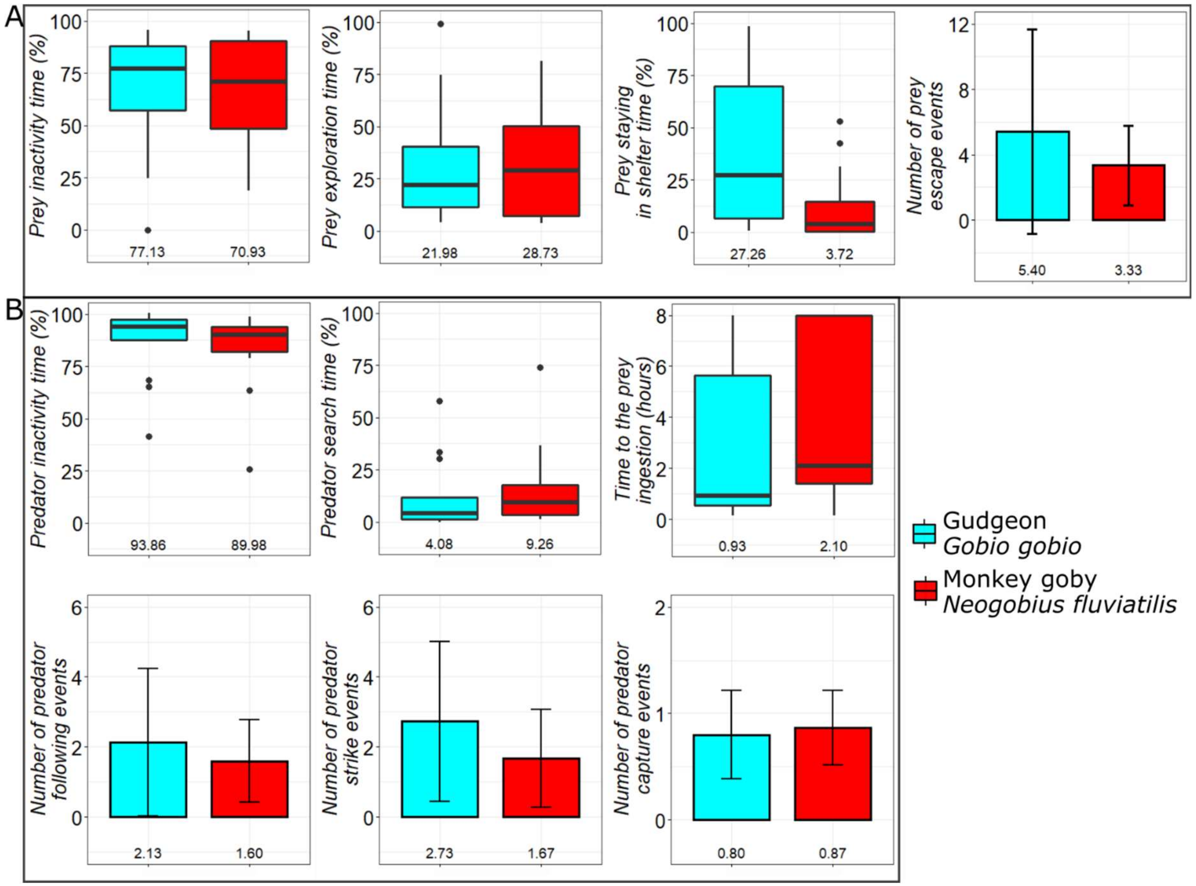


Supplementary Material A: Charts representing raw data for all variables included in PCA for European bullhead/racer goby pair. A – variables analyzed for prey behaviors; B – variables analyzed for predator behavior. Values above the X-axis represent median values for boxplots and mean values for bar plots. Error bars in bar plots correspond to the standard deviation.

Augustyniak et al. (2023) – Supplementary material 2



Supplementary Material B: Charts representing raw data for all variables included in PCA for gudgeon/monkey goby pair. A – variables analyzed for prey behaviors; B – variables analyzed for predator behavior. Values above the X-axis represent median values for boxplots and mean values for bar plots. Error bars in bar plots correspond to the standard deviation.

Publikacja 2

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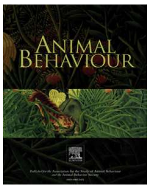

Behavioural responses to environmental novelty in demersal, shelter-associated invasive fish and their native analogues

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Invasive species may differ from native species in terms of behavioural responses to the stress of encountering a novel environment. Learning about the nature of these differences can help us understand the mechanisms of dispersal and success of the alien species in colonized environments. Here, we investigated this topic using two Ponto-Caspian gobies as model species. They constitute a specific group of invasive fish spreading in North America and Europe. They are benthic, territorial fish of low mobility; they are poor swimmers and are strongly associated with shelters. We compared the behaviour of two invasive goby fishes (the racer goby, *Babka gymnotrachelus,* and the monkey goby, *Neogobius fluviatilis*) to that of their native counterparts (the European bullhead, *Cottus gobio*, and the gudgeon, *Gobio gobio*, respectively). We used three laboratory tests to measure boldness–shyness traits: shelter occupancy test, novel object test and open field test. The European bullhead left the shelter later and was less active, and avoided the open field to a greater extent than the racer goby. The gudgeon was more associated with the shelter and novel object than the monkey goby and, in contrast to the monkey goby, decreased its activity in the presence of the novel object and in the open field. All the species were attracted to the vicinity of the novel object. Our study suggests that the invasive Ponto-Caspian gobies are bolder when confronted with structural changes in their environment and have a greater potential to spread across the open bottom, devoid of hiding places, compared to their native analogue species.

Freshwater fish are among the largest groups of animals involved in biological invasions Bernery et al., 2022; Genovesi et al., 2015). There is a growing number of papers on the mechanisms of the spread of invasive freshwater fish species (Bernery et al., 2022; Hayes & Barry, 2008), but many aspects of the process are still not fully understood, including the role of behavioural traits. After establishment, invasive organisms expand their ranges in new environments, which, in the case of mobile animals such as fish, is commonly related to active habitat exploration. The tendency to take risks and explore unfamiliar environments is often related to the organism's dispersal ability (Chapple et al., 2012; Myles-Gonzalez et al., 2015). In general, in their introduced range, invasive animals are associated with a higher level of boldness and habitat exploration, and greater dispersal capabilities compared to both conspecifics remaining in the native range (Myles-Gonzalez et al., 2015; Pintor et al., 2008), and native species encountered in the invaded areas (Juette et al., 2014 and references therein). It is unclear, however, how these behavioural traits are manifested in a specific group of demersal, shelter-associated invasive freshwater fish with burst-and-hold swimming modes, such as invasive gobies (Egger et al., 2021; Tierney et al., 2011). A fish exhibiting this specific swimming behaviour maintains its position on the substrate using its modified pelvic fins (pelvic sucker) and moves forward using brief bursts, after which the fish holds still on the substrate again. Thus, the fish characterized by this type of swimming spend most of their time motionless and therefore we can expect that their behavioural reaction to novelty will be different from that of the fish continuously present in the water column. For burst-and-hold swimmers, behavioural responses to novelty in the environment can be varied and complex depending on their association with a shelter. For example, for organisms less associated with shelters, a structural change in the environment may pose a threat, whereas for shelter users, such a change may indicate an attractive hiding opportunity. On the other hand, shelter-associated burst-

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and-hold swimmers may respond more negatively to unfamiliar locations. Therefore, the interpretation of boldness and dispersal abilities in these fish is not easy and cannot be inferred from studies on invasive pelagic fish species showing prolonged swimming (e.g. Ashenden et al., 2017; Lukas et al., 2021; Rehage & Sih, 2004). Importantly, it is unclear here how such invasive species differ from their native counterparts in this respect.

The Ponto-Caspian gobies (Gobiidae) provide a good example of typically benthic invasive fish that spread in freshwater systems. Their native Ponto-Caspian region, consisting of the Black, Azov and Caspian Seas, lower sections of rivers flowing into these seas and their deltas, is a rich hotspot of invasive species that have successfully established themselves in many temperate regions of the world. This has been facilitated by their evolution under conditions of high fluctuations of abiotic factors (e.g. salinity, water level, temperature, dissolved oxygen) leading to the formation of a specific assemblage of eurytopic species tolerant to sudden changes in the environment (Bij de Vaate et al., 2002; Rewicz et al., 2014). The Ponto-Caspian species spread successfully within European waters (Roche et al., 2013), facilitated by interconnecting river basins and shipping, including the use of ship ballast waters (Bij de Vaate et al., 2002). Via ballast waters, they have also colonized the Laurentian Great Lakes of North America (Kornis et al., 2012). Invasion of the gobiids has caused changes in biocenoses, as these animals displace or threaten native fish species (Kornis et al., 2012; van Kessel et al., 2016) by outcompeting them for food (Kakareko et al., 2013) or shelter (Błońska et al., 2016; Jermacz et al., 2015), alter food webs as predators (Barton et al., 2005; Janssen & Jude, 2001) and prey (Almqvist et al., 2010) and affect fish parasite populations (Ondračková et al., 2021). The gobies are habitat generalists associated with diverse types of bottom habitats, ranging from homogeneous open bottom areas (Płąchocki et al., 2020; Sapota, 2004) to structurally complex locations (Borcherding et al., 2013; Janáč et al., 2018).

Our goal was to determine whether the behavioural reactions of the invasive gobies to novel environments and risky situations differ from those exhibited by the native co-occurring fish. We conducted laboratory tests to assess a range of behaviours associated with exploration and reactions to novelty in the environment. Three types of exploratory behaviour are worth mentioning here. The first is novelty-adjustive behaviour, in which the animal is passively confronted with a novel environment (an open field) or a novel environmental feature (a new object) and must adapt to the new situation. The second type is novelty-seeking behaviour. In this case, the individual searches for novelty on its own initiative (e.g. by leaving the shelter and exploring the area outside). The last type is goal-oriented, novelty-seeking behaviour. In this case, active exploration is only a means to achieve a goal, which, in itself, may be familiar (e.g. exploring an unfamiliar area to find a familiar food; Mc Reynolds, 1962). In our study, we used behavioural tests to explore both forced and spontaneous exploration, and our laboratory conditions allowed the use of completely novel stimuli (Berlyne, 1960). Following the classical approach of measuring animal boldness–shyness, we performed (1) a shelter occupancy test, (2) a novel object test and (3) an open field test.

We hypothesized that the invasive gobies would react differently to the novel environment compared to their native counterparts. Specifically, we predicted the gobies would be bolder, that is, spend less time in the shelter, approach the novel object sooner and spend more time in the open field, and more explorative, thus covering a greater area in the open field test and leaving the shelter more often. Testing these hypotheses would help identify behavioural traits of strictly benthic fish allowing a better understanding of their dispersal mechanisms, which is particularly important in the case of invasive species.

METHODS

Animals

We tested two goby species of Ponto-Caspian origin, pairing them with their native counterparts, which were local benthic fish, co-occurring with the gobies in the field and with a similar lifestyle: (1) the invasive racer goby, *Babka gymnotrachelus*, and the native European bullhead, *Cottus gobio* and (2) the invasive monkey goby, *Neogobius fluviatilis* and the native gudgeon, *Gobio gobio* (Kakareko et al., 2016; Płąchocki et al., 2020).

We collected ca. 120 juvenile fish (ca. 5 cm in total length) from the wild in summer (July–August). The European bullhead and the racer goby were caught from the Brda River (central Poland, 53°08′52.5′N, 17°58′10.5′E) by a diver using an aquarium net. The gudgeon and the monkey goby were obtained from the Pilica River (eastern Poland, 51°45′49.0″N, 21°08′56.7″E) by electrofishing (EFGI 650, BSE Bretschneider Spezialelektronik, Germany). As we compared the results only between the co-occurring pair members, different capture methods did not influence the results of the comparisons. After capture, we transported the fish to the laboratory and held them in 420-litre single-species stock tanks (50 individuals per tank). The stock tanks were filled with conditioned (24 h aged, aerated) tap water (pH 8.12 ± 0.19, electrical conductivity 606.2 ± 3.7 μS/cm, oxygen level 8.2 ± 0.4 mg/litre and 82.4 ± 3.6%; mean ± SD; measured with a Multi 340i Meter, WTW, Weilheim, Germany) at a temperature of 16.5 ± 0.5 °C, maintained by air conditioning. The stock tanks were equipped with aquarium filters, aerators and ceramic and stony shelters, but had no bottom substrate. The photoperiod was set at a 12:12 h light:dark cycle with lights on at 0700. We fed the fish daily with frozen chironomid larvae ad libitum and exchanged water in the stock tanks once a week (ca. 30% of water volume).

General Experimental Conditions

We performed three laboratory tests to assess behavioural responses of fish to a novel environment: (1) a shelter occupancy test, (2) a novel object test and (3) an open field test. In the shelter occupancy test, the main measure of boldness is the latency to leave the shelter: the shorter the time, the bolder the fish (Brown et al., 2007). In the novel object test, bolder fish tend to inspect the object sooner, more often and spend more time near the object (Wright et al., 2006). In the open field test, boldness is indicated by fish activity, time spent in the central part and the number of entries to the central part of the open field (Collier et al., 2017).

We tested single fish in 33-litre plastic experimental tanks (39 × 30 cm and 28 cm high) with opaque white walls. The tanks were oval to avoid fish using corners as shelters. They were filled with 24 h aged and aerated (with an air stone) tap water. Above the experimental tank, we placed an IP video camera (SNB-6004P, Samsung, Changwon, South Korea) and an infrared lamp (MFL-I/LED5-12 850 nm, eneo, Rödermark, Germany). The whole set-up was covered by Styrofoam screens to prevent fish being disturbed by external visual stimuli. All the test species are nocturnal (Erős et al., 2005; Grabowska et al., 2016; Nowak et al., 2019), spending most of the day hiding against predators. Exploration of a novel environment may put an animal at risk of encountering a predator regardless of the time of the day, but during a dark night the risk may be lower because of the worse prey visibility. Thus, we checked whether the studied species would show different propensities to take this risk during their activity and resting periods, to get a full picture of their behaviour. Therefore, experimental trials were performed both during the day and at night. The trial timing was adjusted to the photoperiod in which the fish were kept and acclimatized to avoid disturbing their circadian rhythm. Accordingly, the experiments were started 1 h after the light was turned on or off for the day and night trials, respectively. At the end of the trial, the fish was moved to a postexperimental tank to guarantee that each fish was used only once in the experiment. The water in the experimental tank was exchanged after each trial. Fish were not fed for 12 h before the trial to standardize hunger levels.

Videos were analysed using Noldus Ethovision XT 10.1 (Noldus Information Technology, Wageningen, The Netherlands). The behaviours determined in the three experiments are listed in Table A1. As the tested species are associated with the bottom of water bodies, we did not take their vertical movements into account. We measured the total length of the fish using the video frames and the ImageJ (Schneider et al., 2012) program to check for natural differences in size between sympatric species (see Results for details).

Experimental Procedures

Shelter occupancy test

The experimental tank was filled with water to the level of 8 cm and a shelter was provided for the fish. This was made of two PVC tubes of different diameters. The smaller tube was glued to the bottom of the tank with aquarium silicone glue, whereas the bigger tube was movable and allowed us to open and close the entrance to the shelter. Additionally, we used two square PVC plates (10 × 10 cm) to prevent the fish from entering the space behind the shelter (Fig. 1a). The shelter was equipped with a removable cap to isolate fish from external stimuli. At the start of the trial, a single fish was placed inside the closed shelter and the cap was placed on the top. After 2 min, the experimenter gently turned the bigger tube to open the shelter and allow the tested fish to swim outside. Each trial lasted for 30 min.

For the video analysis, we defined four zones: (1) the Shelter itself; (2) Inspection Zone, a 2 cm wide ring around the shelter entry; in this zone only the fish's head was outside the shelter; (3) Near Shelter Zone, a 3 cm wide ring around the Inspection Zone; when the fish entered this zone, its whole body was outside the shelter; (4) Dangerous Zone, the rest of the tank bottom (Fig. 2a); a fish was defined as having entered this zone when its head and pelvic fins were inside (here and for the other tests).

Novel object test

The experimental tank was filled with conditioned tap water to the level of 10 cm. A single fish was placed in the experimental tank for 12 h of acclimation. We divided the fish into two treatments: the control group, which had no contact with the novel object (to determine the baseline behaviour of tested fish) and the group experiencing the novel object during the test (hereafter: object group). In the latter group, after the acclimation period, the novel object (made of brown PVC, spherical with bevelled bottom and top; Fig. 1b) was gently dropped from the surface as close to the

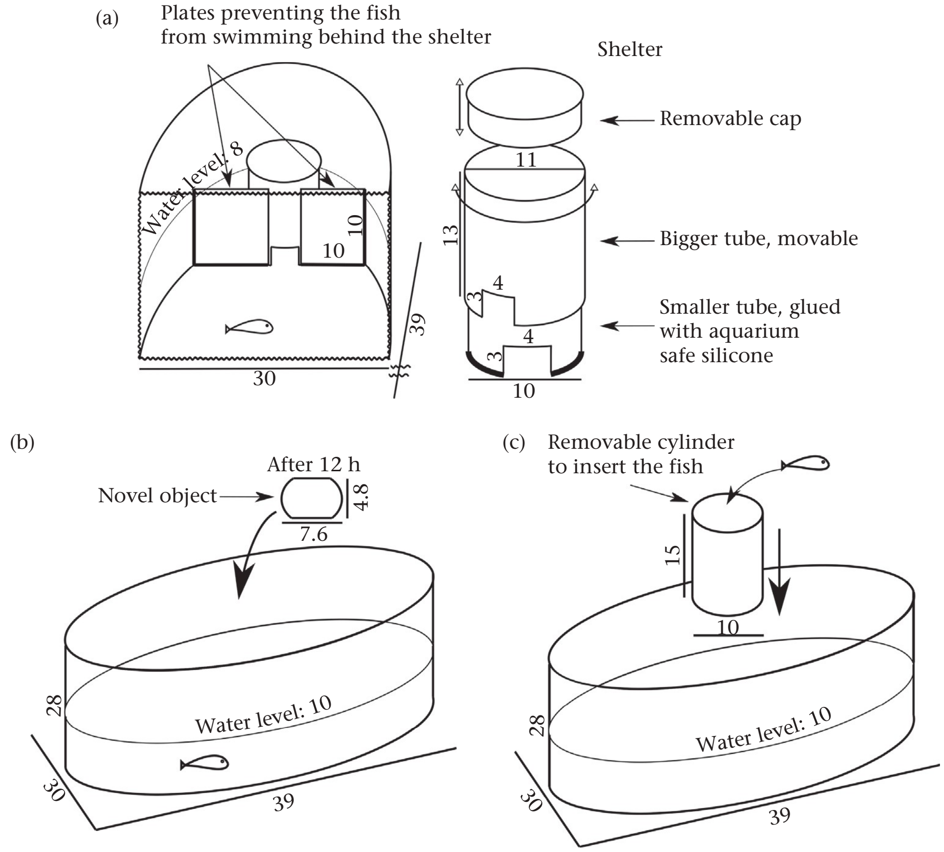

Figure 1. Experimental set-ups. (a) The shelter occupancy test. The fish was placed inside a closed shelter. After 2 min of acclimation, the shelter was opened (the bigger tube was gently turned to match the entrance position with the smaller tube) and the observation started. (b) The novel object test. The fish was placed in the tank for a 12 h acclimation period. Then, in the novel object treatment, the novel object was gently placed near the middle of the tank and the observation started. (c) The open field test. The fish was placed in the insertion cylinder in the tank for a 5 min acclimation. Then, the cylinder was gently removed and the observation started. All the dimensions are given in cm.

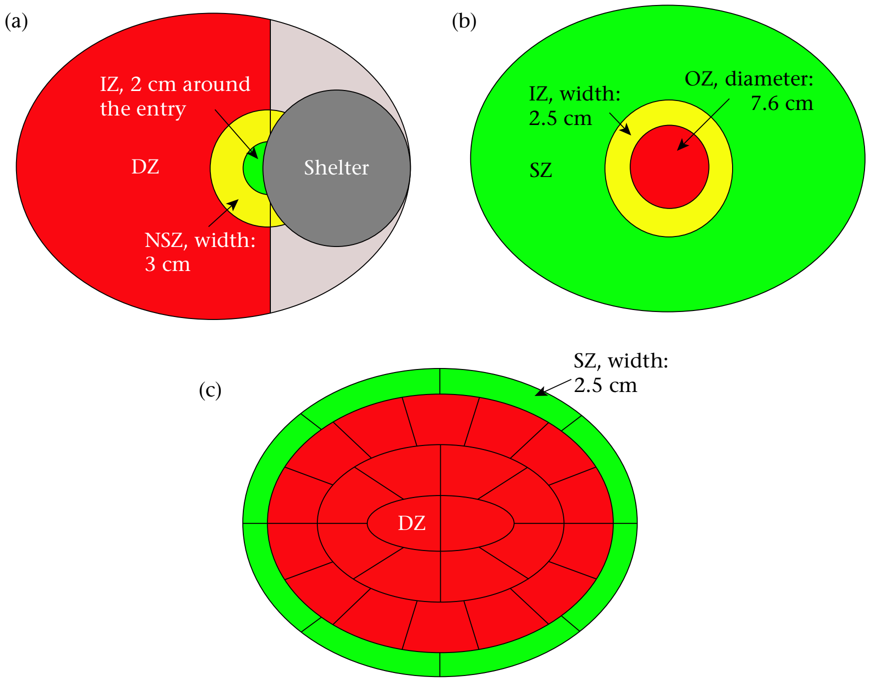

Figure 2. The zones set for the video analysis. (a) The shelter occupancy test. In the Inspection Zone (IZ) only the fish's head was outside the shelter. The fish present in the Near Shelter Zone (NSZ) left the Shelter with its whole body. The rest of the bottom was the Dangerous Zone (DZ). (b) The novel object test. The Object Zone (OZ) was the area of the object itself. In the control group (novel object absent), the Object Zone was set randomly near the centre of the tank, i.e. within the area where the actual novel object sank to the bottom. The Inspection Zone (IZ) was set directly around the OZ. The rest of the bottom was the Safe Zone (SZ). (c) The open field test. The Safe Zone (SZ) contained 25% of the total experimental area (bottom of the tank). The rest of the bottom was the Dangerous Zone (DZ). Both zones were divided into smaller segments of equal areas.

centre of the tank as possible. The novel object was filled with plasticine to make it sink faster and to stabilize it on the bottom. Each trial lasted 20 min after the introduction of the novel object.

The first zone set for video analysis was the Object Zone. This was the part of the bottom covered directly by the object. In the control group, it was a randomly selected place near the centre of the experimental tank, that is, within the area where the actual novel object sank to the bottom. We used this approach to compare fish behaviour after the object introduction to their normal behaviour in the tank. Around the object, we set a 2.5 cm wide (approximately half of the average body length of the tested fish) Inspection Zone. The rest of the tank bottom was the Safe Zone (Fig. 2b).

Open field test

In the open field test, the experimental tank was filled with water to the level of 10 cm. A grey PVC tube (10 cm in diameter, 15 cm in height) was placed in the centre of the experimental tank (Fig. 1c) to provide the same start point for each trial. At the start of the trial, a single fish was placed into the tube for a 5 min acclimation period. Then, the tube was gently removed, and the trial started. Each trial was recorded for 3 h. We selected two test periods from each trial for further analysis. The first period (early response period) was the initial 20 min of the test, which corresponded to the most stressful period for the fish (i.e. just after placing it in the novel environment). The second period (late response period) was between 160 and 180 min after the start of the test. It was set after preliminary observations as a period when the fish became better acclimatized to the novel conditions. The differences between these periods indicate a stress response of the fish to the novel environment.

For the video analysis, we divided the bottom of the tank into 32 segments of equal areas, grouped in two zones. The Dangerous Zone was the central part of the bottom (24 segments). The Safe Zone was the 2.5 cm wide ring along the walls (eight segments; Fig. 2c). We used segments to check whether the fish explored the whole tank bottom or was active only within its limited area.

Ethical Note

The present study adheres to the ASAB/ABS guidelines for the use of animals in research (ASAB Ethical Committee & ABS Animal Care Committee, 2019). The fish were collected and used under the permit of the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland, statement no. 53/2022. In addition, the capture and use of the European bullhead, which is protected by law in Poland, was approved by the Regional Directorate for Environmental Protection in Poland (approval numbers: WOP.6401.4.5.2017.MO, WOP.6401.4.19.2018.MO). All procedures carried out within the study met the European Union guidelines on the protection of animals used for scientific purposes (Directive 2010/63/UE).

Fish were handled carefully during all procedures, including collection from the wild, transport, holding in tanks, testing, etc., to avoid affecting their welfare and behaviour. The catch was made as quickly and gently as possible. The fish were transported (over 2–3 h) in sealed plastic bags filled with oxygenated water, placed inside a Styrofoam cooler to maintain a constant temperature. The housing conditions guaranteed good animal welfare, which was manifested by the overall activity and food intake by the fish throughout the research period. We also did not notice any external signs of stress or disease (e.g. unnatural body shape, skin changes, swimming problems). After the experiments, the European bullhead and gudgeon were released where they were caught. Invasive gobies were euthanized by an overdose of tricaine methane sulphonate (MS-222). We kept fish in the anaesthetic solution up to

10 min after cessation of opercular movement, which is enough to cause brain death (Neiffer & Stamper, 2009).

Statistical Analysis

The differences in fish total length within each pair were tested using paired *t* tests.

For the European bullhead/racer goby pair we performed 16 trials of the shelter occupancy test with the racer goby and 20 trials with the European bullhead for each time of day, and 16 trials for each species × time of day combination in the open field test. In the novel object test, we performed 10 trials with the racer goby for each treatment × time of day combination. For the European bullhead, this experiment was replicated 12 times for each treatment × time of day combination except the treatment with the novel object present during the day, for which 16 trials were performed. For the gudgeon/monkey goby pair, we performed 16 trials of the shelter occupancy test for each species × time of day combination, 12 trials of the novel object test for each species × treatment × time of day combination and 16 trials of the open field test for each species × time of day combination. We compared results only within the above-mentioned pairs, as they were composed of species co-existing and interacting with each other in the same environments.

We performed a principal component analysis (PCA) on the correlation matrix separately for each test and each pair of fish species (six analyses in total) using behavioural variables from Table A1 (except for latency variables in the open field test, see below). The PCA reduces problems with multiple statistical comparisons and avoids the multicollinearity of multiple independent variables by producing orthogonal components based on sets of intercorrelated raw variables. Principal components (PC) were extracted based on their eigenvalues greater than 1. We took the original variables with absolute values of their loadings greater than 0.5 after Kaiser–Varimax rotation into account when explaining the meanings of the particular PCs.

The normality and homoscedasticity assumptions of determined PCs were not violated based on visual inspection of residual plots; thus, we decided to use parametric tests for their further analysis. For the shelter occupancy test, we used a two-way general linear model (GLM), with Species (one of the two species in the pair) and Time of day (day or night) as between-subject factors. Principal components from the novel object test were analysed using a three-way GLM with Species, Time of day and Treatment (control/object) as between-subject effects. For this test, we only interpreted terms involving the novel object presence effect as indicating responses of fish to the introduction of the novel object. For the open field test, we used a three-way general linear mixed model (GLMM), where Species and Time of day were between-subject fixed effects, Period (early or late response) was a within-subject fixed effect, and an individual ID was a random effect. For the open field test, we analysed the latency variables (time to the first occurrence of an event) separately, using the Cox proportional hazards model, as censored observations were present in the data set. Moreover, these variables were valid only for the early response period. For the novel object and shelter occupancy tests, we included latency variables to the PCA. For each analysis, we started with the full factorial model, then we simplified it by dropping consecutively the highest order nonsignificant interaction terms. Based on the Akaike information criterion (AIC), we retained a more complex model when its AIC was lower than that of the simpler model by 2 or more (Burham & Anderson, 2002). All models were then followed by Tukey HSD post hoc tests. For the novel object test, we were interested only in fish responses to the introduction of the novel object to the environment; thus, we only interpreted the main effect of Treatment and its interactions with the other factors. In the case of interactions, we tested all comparisons involving treatment.

All the analyses were run in R 4.1.1 (R Core Team, 2021). The Cox proportional hazards model was run using the 'survival' package (Therneau, 2022), GLMMs using the 'lmerTest' package (Kuznetsova et al., 2017) and post hoc tests using the 'emmeans' package (Lenth, 2022).

RESULTS

The descriptive statistics (means, SDs, sample sizes) for behaviours measured during each experiment are available in the Supplementary material.

The total length of the fish was (mean ± SD) 5.24 ± 0.75 cm for the European bullhead, 5.30 ± 0.78 cm for the racer goby, 4.93 ± 0.93 cm for the gudgeon and 4.44 ± 0.82 for the monkey goby. It did not differ between the species for the European bullhead/racer goby pair (paired *t* test: open field test: $t_{31} = -0.65$, $P = 0.518$; novel object test: $t_{39} = -0.82$, $P = 0.419$; shelter occupancy test: $t_{13} = 0.70$, $P = 0.494$). For the gudgeon/monkey goby pair, the gudgeon individuals were longer than the monkey goby individuals (paired *t* test; open field test: $t_{31} = 2.11$, $P = 0.043$; novel object test: $t_{46} = 3.62$, $P < 0.001$; shelter occupancy test: $t_{22} = 2.43$, $P = 0.024$). As the fish in both pairs lived in sympatry, we considered that the size differences between the gudgeon and the monkey goby reflected those found in nature.

The PCA extracted in total nine PCs for the racer goby/European bullhead pair (two PCs for the shelter occupancy test, four PCs for the novel object test, three PCs for the open field test) and 10 PCs for the gudgeon/monkey goby pair (three PCs for the shelter occupancy test, four PCs for the novel object test, three PCs for the open field test). The details of the PCA results are presented in Tables A2 and A3.

The European Bullhead/Racer Goby Pair

In the shelter occupancy test, high PC1 scores indicated low exploration of the area outside the shelter (shyness; Table A2). The GLM for PC1 showed a significant effect of Time of day (Table A4). Both species explored the area outside the shelter more at night than during the day (Fig. 3a). For PC2, high scores indicated fewer shelter exits and a higher association with the shelter (fear; Table A2). The GLM showed a significant effect of a Species*Time of day interaction (Table A4). The European bullhead tested during the day was more associated with the shelter than at night, as well as more associated with the shelter compared to the racer goby (Fig. 3b).

In the novel object test, higher PC1 scores indicated more time spent in the Inspection Zone, that is, at some distance to the object, rather than in its close proximity (Table A2). The GLM for PC1 showed a significant effect of the Species*Time of day*Treatment interaction (Table A5). When tested during the day, both species spent time in the Inspection Zone experiencing the novel object. At night, only the racer goby exhibited this response (Fig. 4a). For PC2, higher scores indicated low general activity (inactivity: Table A2). The GLM showed a significant Species*Time of day*Treatment interaction (Table A5). When tested at night, both species decreased their activity after the appearance of the novel object compared to their baseline behaviour. This activity reduction shown by the European bullhead was more pronounced than that shown by the racer goby as indicated by the significant difference in activity level between species at night when the novel object was present. Moreover, in the presence of the novel object, the European bullhead was less active at night than during the day (Fig. 4b).

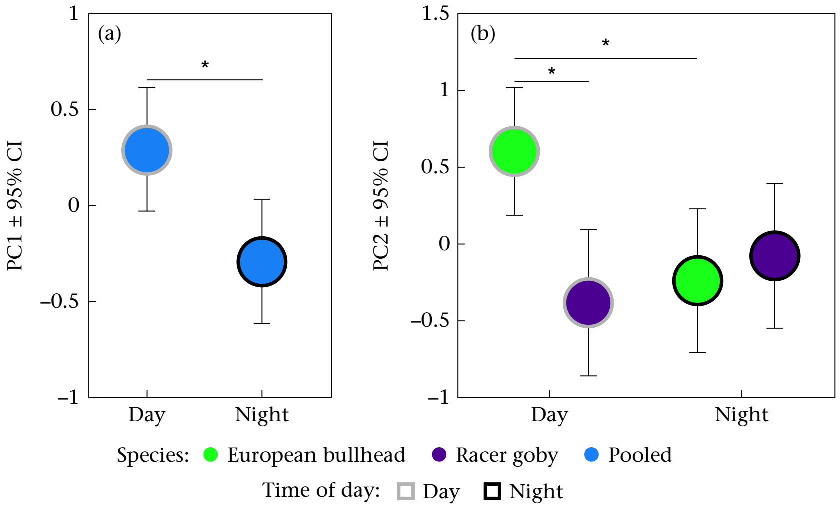

Figure 3. Shelter occupancy by the European bullhead and racer goby depending on the time of day (day, night): (a) principal component 1 (PC1) indicates a low level of exploration of the area outside the shelter and (b) PC2 indicates a high association with the shelter (Table A2). The presented values are estimates (means ± 95% confidence interval) predicted for significant terms of the general linear models (Table A4): a main effect of Time of day (day/night) (a) and Time of day*Species (bullhead/racer goby) interaction (b). Asterisks indicate differences at $P < 0.05$.

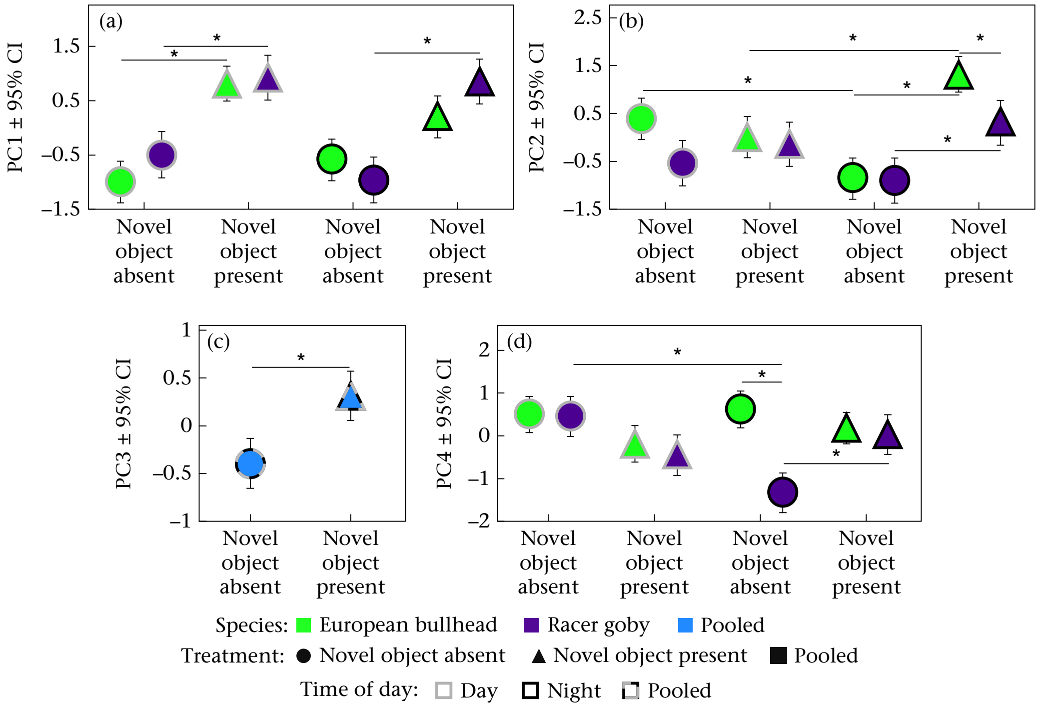

Figure 4. Responses of the European bullhead and racer goby to the novel object: (a) principal component 1 (PC1) indicates Inspection Zone use, (b) PC2 indicates inactivity, (c) PC3 indicates a low number of freezing events and (d) PC4 indicates object avoidance (Table A2). The presented values are estimates (means ± 95% confidence interval) predicted for significant terms of the general linear models (Table A5): (a, b, d) Species (bullhead/racer goby)*Treatment (novel object absent/present)*Time of day (day/night) interaction and (c) a main effect of Treatment. Asterisks indicate differences at $P < 0.05$.

For PC3, higher values indicated fewer freezing events by the fish (Table A2). The GLM showed significant main effects of Species and Treatment and a Species*Time of day interaction (Table A5). Both species exhibited more freezing events in the absence of the novel object than in its presence (Fig. 4c). For PC4, higher scores indicated avoidance of the zone directly associated with the object (Table A2). The GLM showed a significant Species*Time of day*Treatment interaction (Table A5). The racer goby avoided the novel object at night. (Fig. 4d).

For PC1 in the open field test, higher scores indicated open field exploration (Table A2). The GLMM showed a significant effect of Species, as well as Species*Period and Time of day*Period interactions (Table A6). The European bullhead decreased its open field use in the late response period compared to the early response period. In contrast, the racer goby increased its open field use in the late response period compared to the early response period. During the late response period, the racer goby used the open field more often than the European bullhead (Fig. 5a). The racer goby used the open field more at night than during the day. Moreover, at night, it used the open field more than the European bullhead (Fig. 5b). For PC2, higher scores indicated high general activity level (Table A2). The GLMM showed significant main effects of Species and Period, as well as of their interaction, and of a Species*Time of day interaction (Table A6). The European bullhead was more active than the racer goby during the day (Fig. 5c). In the early response period, the European bullhead was more active than in its late response period and than the racer goby (Fig. 5d). For PC3, higher scores indicated exploration of the safe, peripheral part of the arena (Table A2). The GLMM showed significant main effects of Species, Period and their interaction (Table A6). The European bullhead explored the Safe Zone to a greater extent than the racer goby during the early response period. The racer goby explored the Safe Zone to a lesser extent during the early than in the late response period (Fig. 5e). The Cox proportional hazards model showed a significant effect of Species on the latency to the first movement and the first transition from the Safe Zone to the Dangerous Zone (Table A7). The European bullhead started both activities sooner than the racer goby (Fig. 6).

The Gudgeon/Monkey Goby Pair

In the shelter occupancy test, the higher scores of PC1 indicated low exploration of the area outside the shelter (shyness; Table A3). The GLM showed significant main effects of Species and Time of day (Table A8). The gudgeon explored the area outside the shelter to a lesser extent than the monkey goby (Fig. 7a). Both species explored this part of the arena less during the day than at night (Fig. 7b). On the other hand, the GLM showed no significant effects of the tested factors on PC2 (Table A8), for which higher scores indicated fewer shelter exits and a higher association with the shelter (fear; Table A3). For PC3, higher scores indicated more time outside the shelter (Table A3). The GLM showed a significant effect of Time of day (Table A8): both species spent more time outside the shelter at night than during the day (Fig. 7c).

In the novel object test, higher scores of PC1 indicated high general activity of the fish (Table A3). The GLM showed a significant main effect of Time of day and of a Species*Treatment interaction (Table A9). The gudgeon decreased its activity after the novel object appearance. Moreover, when the object was present in the arena, the gudgeon was less active than the monkey goby (Fig. 8a). For PC2, higher scores indicated object avoidance (Table A3). The GLM showed a significant effect of Species*Treatment and Time of day*Treatment interactions (Table A9). Both species spent more time in zones related to the object after the novel object appearance compared to the control. This reaction was more pronounced in the gudgeon, as indicated by the significant difference between the species when the novel object was present (Fig. 8b). Both species preferred the object more during the day than at night (Fig. 8c). For PC3, higher scores indicated few object inspections (visits in zones around the Novel Object; Table A3). The GLM showed significant Species*Treatment and Time of day*Treatment interactions (Table A9). The monkey goby entered the areas near the object less often than in the control and less often than the gudgeon (Fig. 8d). Moreover, at night, the fish visited the novel object zones less compared to their baseline behaviour (Fig. 8e). For PC4, higher scores indicated more freezing events and more time spent in the Safe Zone (fear; Table A3). The GLM showed significant effects of

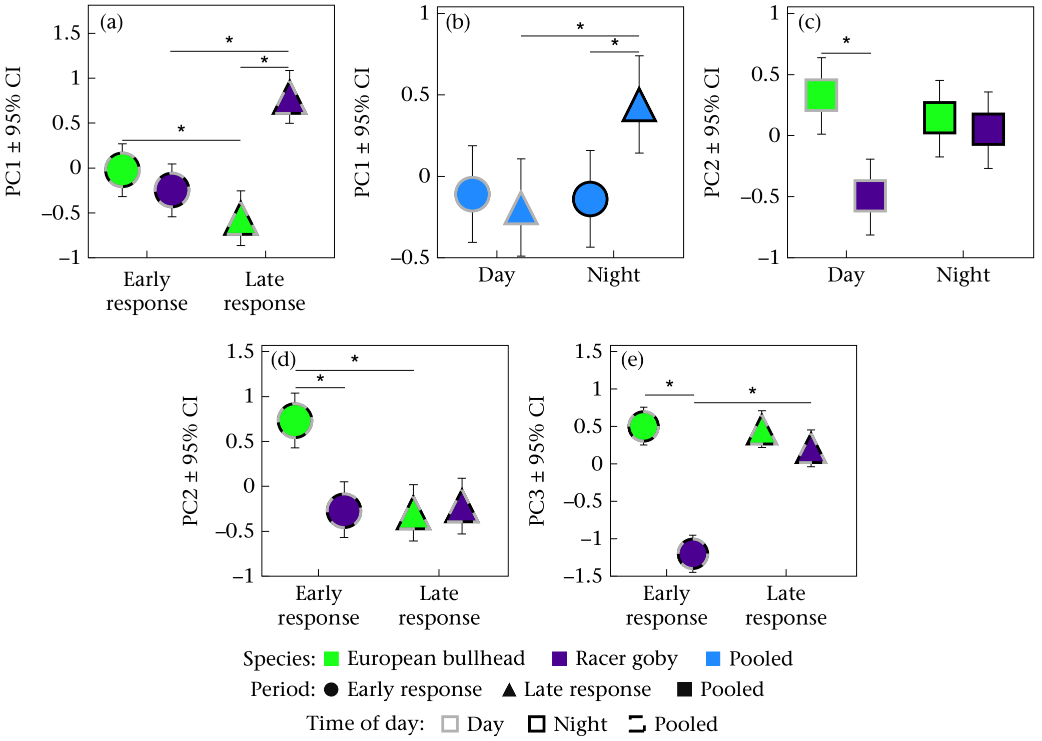

Figure 5. Behaviours of the European bullhead and racer goby in the open field test: (a, b) principal component 1 (PC1) indicates open field exploration, (c, d) PC2 indicates activity and (e) PC3 indicates exploration of the peripheral part of the arena (Table A2). The presented values are estimates (means ± 95% confidence interval) predicted for significant terms of the general linear mixed models (Table A6): (a, d, e) Period (early/late response)*Species (bullhead/racer goby) interaction, (b) Time of day (day/night)*Period interaction and (c) Time of day*Species interaction. Asterisks indicate differences at $P < 0.05$.

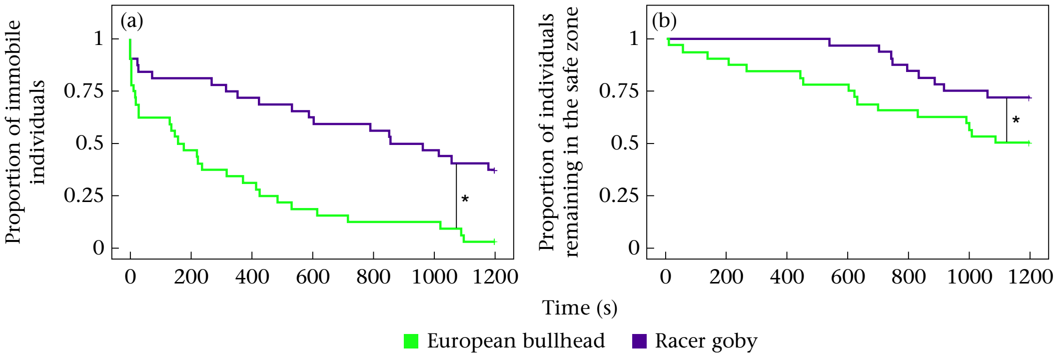

Figure 6. The latency to (a) the first movement and (b) the first transition from the Safe Zone (SZ) to the Dangerous Zone (DZ) of the European bullhead and racer goby after being introduced to the open field tank. The presented values are predicted by the Cox proportional hazards model for species differing significantly from each other (as shown by asterisks), after pooling day and night responses (nonsignificant effect of Time of day; Table A7).

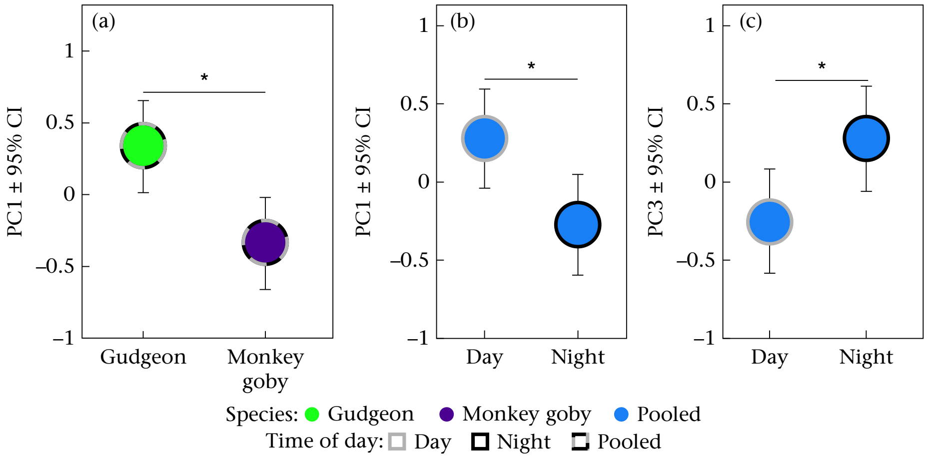

Figure 7. Shelter occupancy by the gudgeon and monkey goby depending on Time of day (day, night): (a, b) principal component 1 (PC1) indicates low exploration of the dangerous area and (c) PC2 indicates time outside the shelter (Table A3). The presented values are estimates (means ± 95% confidence interval) predicted for significant terms of the general linear models (Table A8): (a) a main effect of Species (gudgeon/monkey goby) and (b, c) Time of day (day/night). Asterisks indicate differences at $P < 0.05$. For PC2, no significant effects of the tested factors were found in the model.

Species*Time of day, Species*Treatment and Time of day*Treatment interactions (Table A9); however, the post hoc tests revealed the differences only for the last interaction. During the day, both species decreased their fear level after the novel object appearance compared to their baseline behaviour. At night, we observed the opposite response: in the presence of the novel object, both species increased their fear level compared to the baseline behaviour. In general, both species exhibited more fear responses at night than during the day when the novel object was present (Fig. 8f).

For PC1 in the open field test, higher scores indicated low activity and more freezing events (inactivity; Table A3). The GLMM for PC1 showed a significant effect of a Species*Time of day*Period interaction (Table A10). In the early response period, the gudgeon was less active than the monkey goby regardless of the time of day. In the late response period, the gudgeon was also less active during the day than at night. Moreover, at night, the gudgeon was less active in the early than in the late response period (Fig. 9a). For PC2, higher scores indicated high explorative behaviour (Table A3). The GLMM showed a significant effect of a Species*Period interaction (Table A10). The monkey goby was more explorative in the early than in the late response period (Fig. 9b). For PC3, higher scores indicated more time spent in and more visits to the Dangerous Zone (boldness; Table A3). The GLMM showed a significant effect of a Species*Time of day*Period interaction (Table A10), resulting from the higher boldness of the gudgeon compared to the racer goby in the late response period during the day (Fig. 9c). The Cox proportional hazards model showed a significant effect of Species on the latency to the first movement (Table A11), whereas there were no effects of the tested factors on the time of the first transition from the Safe Zone to the Dangerous Zone (Table A11). The gudgeon showed the first movement earlier than the monkey goby (Fig. 10).

DISCUSSION

We confirmed our hypothesis that, at least in some contexts shaped by additional factors, the invasive gobies presented different behaviours from their native counterparts when facing novel situations. The results of the three tests, used to assess boldness–shyness of the fish, suggest that the invasive gobies were relatively bolder than the natives.

In the shelter occupancy test, the European bullhead left the shelter later and was less active outside the shelter than the racer goby. The gudgeon also showed higher affinity with the shelter and explored the arena outside the shelter to a lesser extent than the monkey goby. We therefore observed a similar pattern of behavioural differences in the pairs of species studied. In both cases, the gobies were less shelter-oriented (although for the racer goby this was visible only during the day) and more active than the natives.

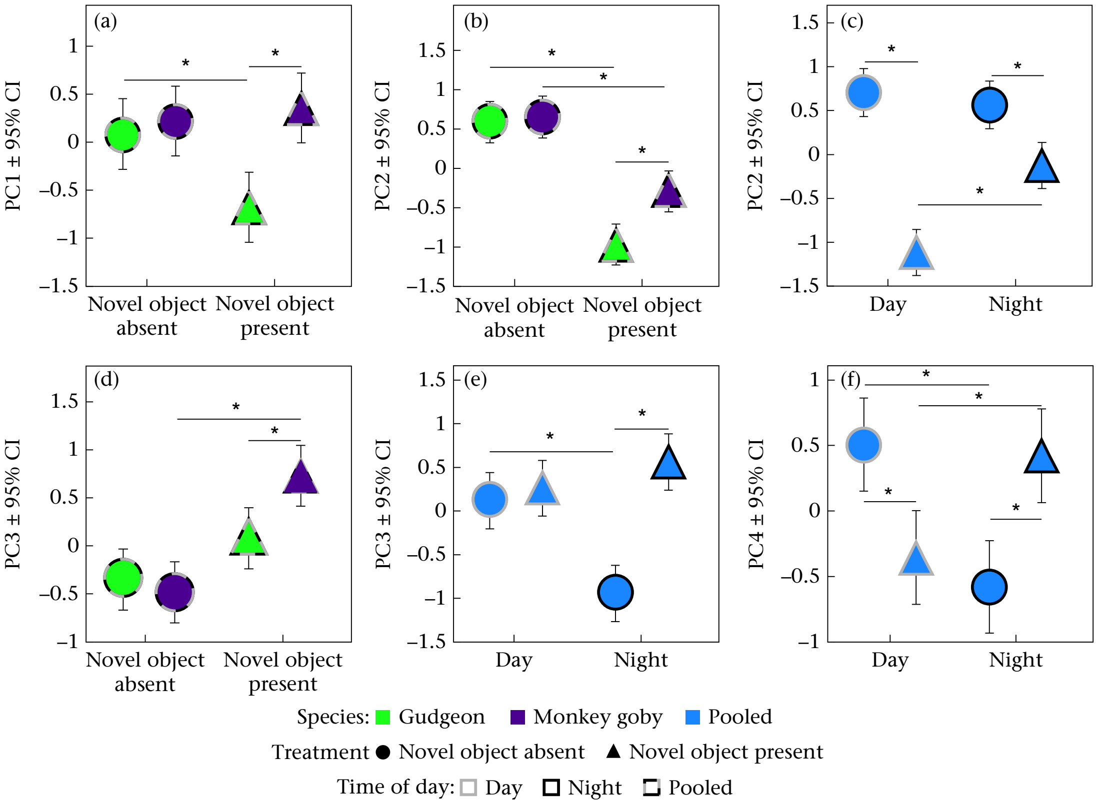

Figure 8. Responses of the gudgeon and monkey goby to the novel object: (a) principal component 1 (PC1) indicates activity, (b, c) PC2 indicates object avoidance, (d, e) PC3 indicates a low number of object inspection events and (f) PC4 indicates fear (Table A3). The presented values are estimates (means ± 95% confidence interval) predicted for significant terms of the general linear models (Table A9): (a, b, d) Species (gudgeon/monkey goby)*Treatment (novel object absent/present) interaction and (c, e, f) Time of day*Treatment interaction. Asterisks indicate differences at $P < 0.05$.

These behaviours in the shelter occupancy test are typically considered boldness indicators (Brydges & Braithwaite, 2009; McCormick et al., 2017; Mustafa et al., 2019). In this context, the two gobies could be considered bolder, as they were more likely to exhibit active novelty-seeking behaviour and take risks in an area outside their safe hiding place.

In the novel object test, all the species were attracted to the object's vicinity, which suggests that they seemed to treat it as a potential shelter. On the other hand, they tended to decrease their activity in the presence of the object. A decrease in activity in the form of an active search for stimuli is one of the most common reactions of animals to a threat and the observed behaviour fits more to an attempt to adapt to the prevailing conditions. This reaction was generally more pronounced in the native species than in the invaders. Additionally, for the European bullhead and racer goby pair there were differences in fish reaction to the novel object depending on the time of day. This dependence may be explained by the lower visibility of the object at night (i.e. in darkness) compared to the day, and, in consequence, a limited capability of assessment of the real threat. As the novel object in our test was an artificial, inanimate object, the only source of information about it was the visual cue. When the object appeared during the day, both species could see and determine it as not dangerous. When the same situation took place at night, low visibility combined with the absence of other signals (i.e. chemicals) resulted in a higher level of uncertainty about danger for the fish. The propensity to inspect a novel object shown by the racer goby combined with its higher activity, despite its avoidance of the novel object itself, could be associated with the higher boldness (White et al., 2013) of this species at night compared to the European bullhead. The native gudgeon spent more time close to the object and visited the zones around it more often than the monkey goby. These results interpreted alone may indicate that the gudgeon is bolder than the monkey goby as it took a risk inspecting the novel object (White et al., 2013; Wright et al., 2006). However, the higher affinity of the gudgeon with the shelter (in the shelter occupancy test) and its lower general activity after the novel object's appearance compared to the monkey goby suggest that the gudgeon perceived the object as a potential shelter, and that is why it was associated with its vicinity. At the same time, the bolder monkey goby explored the environment.

The results of the open field test, considered in the light of the results of the previous two tests, indicated lower boldness of the native fish compared to the invasive gobies. In the first pair, the European bullhead started its activity faster, was more active and explored the peripheral zone to a greater extent than the racer goby in the initial period after emerging into the open field, suggesting higher boldness (Forsatkar et al., 2016). However, this activity can also be interpreted as an attempt to find an escape route to a more friendly environment in a sheltered location. The results of the shelter occupancy and novel object tests showed that the bullhead was more associated with the shelter and less active after the novel object's appearance than the racer goby. Taking the results of the three tests together, the high levels of activity and exploration expressed by the bullhead during the initial period of the open field test might have resulted from the hyperactivity caused by the high anxiety experienced by the tested fish (Jarrold et al., 2020). As the bullhead was active mostly in the peripheral part of the arena, this

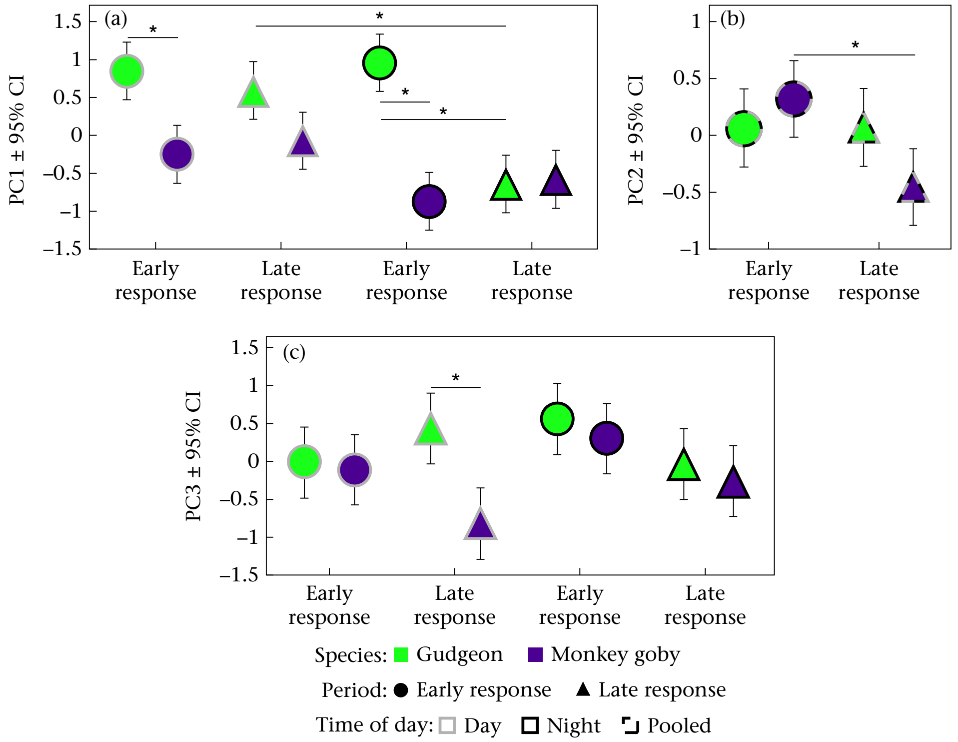

Figure 9. Behaviours of the gudgeon and monkey goby in the open field test: (a) principal component 1 (PC1) indicates inactivity, (b) PC2 indicates explorative behaviour and (c) PC3 indicates boldness (Table A3). The presented values are estimates (means ± 95% confidence interval) predicted for significant terms of the general linear mixed models (Table A10): (a, c) Species (gudgeon/monkey goby)*Time of day (day/night)*Period (early/late response) interaction and (b) Species*Period interaction. Asterisks indicate differences at $P < 0.05$.

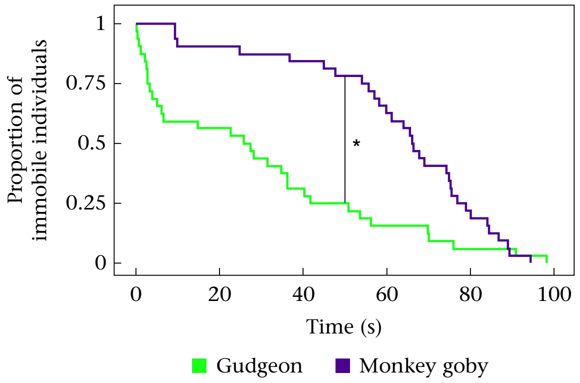

Figure 10. The latency to the first movement of the gudgeon and monkey goby after being introduced to the open field tank. The presented values are predicted by the Cox proportional hazards model for species differing significantly from each other (as shown by the asterisk), after pooling day and night responses (nonsignificant effect of Time of day; Table A11).

was likely related to looking for a refuge and/or possibility to escape. The results from the late response period, when the fish became more acclimated to the experimental arena, support the idea that, apart from an initial period of hyperactivity, the European bullhead was less explorative (which could be interpreted as more cautious) than the racer goby. The exploration of the open field by the European bullhead decreased, while the exploration of the whole arena by the racer goby increased compared to the early response period. As a result, the racer goby during the late response period explored the whole arena to a greater extent than the bullhead, while during the initial period, facing the uncertainty about the environment, the racer goby reduced its activity to avoid detection by a potential predator. To sum up, we are of the opinion that behavioural reactions to stress related to the novel environment were more pronounced in the European bullhead, which is more associated with shelter and less explorative than the racer goby. In the second pair of fish tested, we do not associate the higher activity of fish with hyperactivity, and we therefore interpret it classically as a sign of boldness. This is because there is no contrast between the elevated activity of the fish in the initial phase of the open field test and the generally low activity in the previous tests, as it was in the case of the European bullhead. The native gudgeon showed lower activity in the early response period compared to the late response period at night. Such lower activity in the initial period in the novel environment is a typical behavioural response to danger (Lima & Dill, 1990; Teplitsky & Laurila, 2007), indicating that the gudgeon was less bold than the monkey goby. Moreover, the bolder behaviour of the monkey goby was also confirmed by its higher activity in the initial test period compared to the gudgeon, regardless of the time of day. We observed the lower boldness of the monkey goby compared to the gudgeon in the late response period at night and the lower explorative behaviour of the former in the late response period compared to the initial period regardless of the time of day. In light of the previous, shorter tests, we may assume that the goby collected information about the environment during the initial period in the open field test, so that in the second period of this test it could reduce its activity, as there were no further changes in the environment (no novel stimuli). To summarize, the behavioural responses to novelty expressed by the gudgeon were more pronounced than those of the monkey goby.

Our findings on the activity of these fish and their association with the hiding places, are supported by the other studies that have been carried out on these species. Augustyniak et al. (2022) showed that the European bullhead, facing a predator danger (a direct stressor affecting behaviour), spent more time in the shelter and was less active than the racer goby. The greater association of the European bullhead with the shelter compared to the racer goby was also shown by Grabowska et al. (2016). In the wild, the European bullhead is commonly associated with areas with high water velocities (Carter et al., 2004; Roje et al., 2021) and stony bottoms (Kakareko et al., 2016). Thus, the shelter, besides providing protection against predators, allows them to save energy resources when facing higher water currents. The racer goby, in turn, occurs in more lentic habitats, including soft (mud, sand) sediments (Kakareko et al., 2016; Płąchocki et al., 2020), which demand lower energy expenditure to keep the position against the water current. The more pronounced behavioural responses of the gudgeon than the monkey goby to predation cues was shown by Kłosiński et al. (2022). They found that the gudgeon decreased its activity to a greater extent relative to the monkey goby. Importantly, the gudgeon individuals showed thigmotaxis in the presence of predation cues. The authors concluded that this probably increased the sense of safety in stressed fish. These results can be related to our observations in the novel object test, where the gudgeon spent more time near the object and treated it as a potential shelter. Combining the results by Kłosiński et al. (2022) and our current study, it seems that the gudgeon facing a stressful situation first tries to find a shelter. This seems to be in contrast with the results of Augustyniak et al. (2022), where the gudgeon facing a direct predator danger exhibited escape responses instead of staying inside a provided shelter. However, we could assume that the risk of a direct predator attack is a more stressful stimulus than the novel environment or the presence of an alarm substance alone; thus, the fish exhibit different behavioural strategies to survive. The monkey goby, in turn, relies on the shelter to a lesser extent, as in dangerous situations it may burrow in the sandy bottom and stay there motionless (e.g. Čápová et al., 2008; Erős et al., 2005).

To summarize, in the present work, we showed for the first time that the invasive Ponto-Caspian gobies faced with stressful situations related to sudden changes in the environment containing a new stimulus of an absolute nature (new object, open field) may be less dependent on the availability of hiding places than native species of the same guild. Instead, they explore the area to a greater extent than their native counterparts. Although these differences seem to appear in specific ecological contexts shaped by additional factors, for example, the time of day, they are likely to occur in the wild and affect behaviour. It is also worth noting that the gudgeon was the only gregarious species in our study; thus, the presence of conspecifics could also affect its behavioural responses to novelty. Nevertheless, behaviours exhibited by invasive gobies may enhance their dispersal abilities over long distances, as active exploration of the area may increase the probability of finding the transport vector (e.g. entering the ballast water tank of a ship). This would allow the invaders to enter novel areas (Chapple et al., 2012), whereas their boldness may be related to the short-distance (local) dispersal after their release in the field (Fraser et al., 2001). The same set of behaviours may facilitate goby establishment in the invaded areas, as their greater activity may lead to more efficient resource finding and utilization compared to the native fish species present in the environment. Additionally, the gobies, which are less shelter-dependent than the native species encountered in the invaded areas, may be capable of colonizing bare, open bottom areas, which was not considered earlier. We must keep in mind that the greater boldness of the gobies compared to the natives may also increase the risk of encountering a predator (McGlade et al., 2022), although this is not always the rule as Blake et al. (2018) showed a positive predator-dependent role of boldness in the prey's survival. However, the net effect of such a set of features can significantly increase the chances of a successful invasion of these species in aquatic environments where human interference leads to uniformity of the bottom topography, such as the offshore areas of regulated, channelized rivers. The results of our study also bring new information about the methodology used to study the boldness-related behaviours of shelter-associated species. As the gudgeon in our study treated the novel object as a shelter rather than as a source of novelty, we recommend introducing a familiar hiding place in the experimental set-up of future novel object tests with this species. Moreover, to avoid the hyperactivity of the tested fish in the open field, as shown by the European bullhead in our study, the methodology of the open field test for this specific group of shelter-associated fish species might be improved by providing more realistic conditions inside the arena, by, for example, adding a substrate to the bottom of the tank.

Author Contributions

Mateusz Augustyniak: Conceptualization, Methodology, Investigation, Resources, Formal analysis, Visualization, Writing—Original draft, Writing—Review & editing. **Jarosław Kobak**: Conceptualization, Methodology, Formal analysis, Writing—Original draft, Writing—Review & editing. **Maciej Trojan:** Writing—Original draft, Writing—Review & editing. **Tomasz Kakareko**: Conceptualization, Methodology, Resources, Writing—Original draft, Writing—Review & editing, Supervision, Funding acquisition.

Data Availability

The data set used in this study is available at https://data.mendeley.com/datasets/gps2y3kggs/1.

Declaration of Interest

The authors declare no conflict of interest.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2023.11.008.

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Appendix

Table A1
The list of behaviours determined during the analysis of each test

Shelter occupancy test	Novel object test	Open field test
Continuous events		
Time spent in the Shelter (%ET)	Time spent in SZ (%ET)	Time spent in DZ (%ET)
Time spent in IZ (%)	Time spent in IZ (%ET)	Latency to the first transition SZ-DZ (s)
Latency to the first visit in IZ (s)	Latency to the first visit in IZ (s)	Latency to the first movement (s)
Time spent in NSZ (%ET)	Time spent in the OZ (%ET)	Movement duration (%ET)
Latency to the first visit in NSZ (s)	Latency to the first visit in OZ (s)	Mean duration of freezing events (s)
Time spent in DZ (%ET)	Mean distance to the Object (cm)	
Latency to the first visit in DZ (s)	Latency to the first movement (s)	
Mean distance to the Shelter entry (cm)	Movement duration (%ET)	
	Mean duration of freezing events (s)	
Count events		
Number of Shelter exits	Number of visits in IZ	Unique segments visited in SZ
Number of visits in NSZ	Number of visits in OZ	Unique segments visited in DZ
Number of visits in DZ	Number of freezing events (inactivity >30 s)	Number of visits in DZ
		Number of freezing events (inactivity >30 s)

DZ, Dangerous Zone; SZ, Safe Zone; IZ, Inspection Zone; NSZ, Near Shelter Zone; OZ, Object Zone; %ET, Percentage of the total experimental time.
See Fig. 2 for explanation of zones in the different experiments.

Table A2
Results of the principal component analyses (PCA) on behavioural variables of the European bullhead and racer goby in various experiments.

PC[a]	λ[b]	% Variance[c]	Variable loadings[d]
Shelter occupancy test			
PC1	7.7	70.0	Time in the Shelter (0.843), Distance to the Shelter entry (−0.838), Latency to the first IZ visit (0.526), Time in the NSZ (−0.687), Number of the NSZ visits (−0.802), Latency to the first DZ visit (0.816), Time in the DZ (−0.934), Number of the DZ visits (−0.931)
PC2	1.3	12.2	Time in the Shelter (0.512), Number of the Shelter exits (−0.863), Latency to the first IZ visit (0.786), Time in the IZ (−0.852), Latency to the first NSZ visit (0.782)
Novel object test			
PC1	3.7	31.0	Time in the SZ (−0.844), Latency to the first IZ visit (−0.614), Time in the IZ (0.917), Distance to the OZ (0.730)
PC2	3.1	25.5	Latency to the first movement (0.744), Time on the move (−0.866), Freezing event duration (0.613)
PC3	1.3	10.9	Freezing events number (−0.924)
PC4	1.0	8.6	Latency to the first IZ visit (0.530), Number of the IZ visits (−0.851), Latency to the first OZ visit (0.849), Time in the OZ (−0.709), Number of the OZ visits (−0.524)
Open field test			
PC1	3.1	46.0	Number of the DZ visits (0.832), Unique segments in the DZ (0.770)
PC2	1.3	19.4	Time on the move (0.644), Freezing events number (−0.946)
PC3	1.1	16.2	Unique segments in the SZ (0.754), Time in the DZ (−0.977), Freezing event duration (−0.697)

DZ, Dangerous Zone; SZ, Safe Zone; IZ, Inspection Zone; NSZ, Near Shelter Zone; OZ, Object Zone. See Fig. 2 and Table A1 for details.

[a] Principal components discriminated by the PCA.

[b] Eigenvalue of the principal component.

[c] Percentage of variance explained by the principal component.

[d] Correlations of measured variables with the principal component after Kaiser–Varimax rotation (loadings with absolute values higher than 0.5 shown).

Table A3
Results of the principal component analyses (PCA) on behavioural variables of the gudgeon and monkey goby in various experiments

PC[a]	λ[b]	% Variance[c]	Variable loadings[d]
Shelter occupancy test			
PC1	5.1	46.1	Distance to the Shelter entry (−0.889), Latency to the first IZ visit (0.802), Latency to the first NSZ visit (0.883), Latency to the first DZ visit (0.954), Time in the DZ (−0.813), Number of the DZ visits (−0.694),
PC2	2.9	26.4	Number of the Shelter exits (−0.732), Time in the IZ (−0.826), Time in the NSZ (−0.804), Number of the NSZ visits (−0.689)
PC3	1.0	9.3	Time in the Shelter (−0.932), Number of the Shelter exits (−0.507)
Novel object test			
PC1	3.6	30.0	Latency to the first movement (−0.826), Time on the move (0.617), Freezing event duration (−0.924)
PC2	2.4	20.4	Time in the SZ (0.552), Time in the IZ (−0.835), Time in the OZ (−0.506), Distance to the OZ (0.671)
PC3	1.5	12.8	Number of the IZ visits (−0.864), Latency to the first OZ visit (0.789), Number of the OZ visits (−0.857)
PC4	1.3	10.8	Time in the SZ (0.655), Time on the move (−0.601), Freezing events number (0.868)
Open field test			
PC1	2.2	31.6	Time on the move (−0.900), Freezing events number (0.832)
PC2	1.5	21.1	Unique segments in the SZ (0.876), Unique segments in the DZ (0.815)
PC3	1.3	18.5	Time in the DZ (0.829), Number of the DZ visits (0.670)

DZ, Dangerous Zone; SZ, Safe Zone; IZ, Inspection Zone; NSZ, Near Shelter Zone; OZ, Object Zone. See Fig. 2 and Table A1 for details.

[a] Principal components discriminated by the PCA.

[b] Eigenvalue of the principal component.

[c] Percentage of variance explained by the principal component.

[d] Correlations of measured variables with the principal component after Kaiser–Varimax rotation (loadings with absolute values higher than 0.5 shown).

Table A4
Two-way general linear models to test the shelter occupancy of the European bullhead and racer goby depending on the time of day

Principal component	Effect	*df*	Mean square	*F*	*P*
PC1	Time of day	1	6.09	6.56	**0.013**
Shyness	Residuals	70	0.93		
PC2	Species (S)	1	3.07	3.49	0.07
Fear	Time of day (D)	1	1.99	2.26	0.14
	S*D	1	6.03	6.85	**0.01**
	Residuals	68	0.88		

The Species and Time of day were set as between-subject factors. Bold type indicates significant effects ($P < 0.05$). The analyses were run for principal components obtained in the PCA based on fish behavioural variables (Table A2).

Table A5
Three-way general linear models to test the response of the European bullhead and racer goby to the novel object depending on the time of day

Principal component	Effect	*df*	Mean square	*F*	*P*
PC1	Species (S)	1	0.53	1.22	0.272
Inspection Zone use	Time of day (D)	1	1.34	3.08	0.083
	Treatment (T)	1	48.39	111.43	**<0.001**
	S*D	1	0.13	0.29	0.591
	S*T	1	0.46	1.05	0.308
	D*T	1	0.91	2.10	0.151
	S*D*T	1	2.78	6.39	**0.013**
	Residuals	84	0.43		
PC2	Species (S)	1	6.13	10.93	**0.001**
Inactivity	Time of day (D)	1	0.06	0.10	0.752
	Treatment (T)	1	15.07	26.90	**<0.001**
	S*D	1	0.02	0.04	0.837
	S*T	1	0.00	0.00	0.964
	D*T	1	18.33	32.71	**<0.001**
	S*D*T	1	4.33	7.72	**0.007**
	Residuals	84	0.56		
PC3	Species (S)	1	7.52	9.71	**0.002**
Low number of freezing events	Time of day (D)	1	1.91	2.47	0.120
	Treatment (T)	1	11.06	14.29	**<0.001**
	S*D	1	3.17	4.10	**0.046**
	Residuals	87	0.77		
PC4	Species (S)	1	7.37	10.26	**0.002**
Object avoidance	Time of day (D)	1	0.36	0.50	0.482
	Treatment (T)	1	1.35	1.88	0.174
	S*D	1	4.73	6.58	**0.012**
	S*T	1	3.72	5.17	**0.025**
	D*T	1	7.22	10.05	**0.002**
	S*D*T	1	5.87	8.17	**0.005**
	Residuals	84	0.72		

The Species, Time of day and Treatment (novel object present or absent) were set as between-subject effects. Bold type indicates significant effects ($P < 0.05$). The analyses were run for principal components obtained in the PCA based on fish behavioural variables (Table A2).

Table A6
Three-way general linear mixed model to test the behaviour of the European bullhead and racer goby in the open field depending on the time of day

Principal component	Effect	*df*	Mean square	*F*	*P*
PC1	Species (S)	1	8.80	13.33	**0.001**
Open field exploration	Time of day (D)	1	2.49	3.77	0.057
	Period (P)^{RM}	1	2.03	3.08	0.084
	S*P^{RM}	1	20.12	30.49	**<0.001**
	D*P^{RM}	1	3.59	5.45	**0.023**
PC2	Species (S)	1	6.92	8.64	**0.004**
Activity	Time of day (D)	1	1.08	1.34	0.249
	Period (P)^{RM}	1	7.86	9.81	**0.002**
	S*P^{RM}	1	4.25	5.31	**0.023**
	D*P^{RM}	1	9.18	11.47	**0.001**
PC3	Species (S)	1	31.55	62.59	**<0.001**
Exploration of the peripheral part of the arena	Period (P)^{RM}	1	15.49	30.73	**<0.001**
	S*P^{RM}	1	17.20	34.12	**<0.001**

Species and Time of day were set as between-subject fixed effects, Period (early response, immediately after the introduction to the open field tank, and late response, after the initial acclimation) as a within-subject (repeated measures) fixed effect and fish ID as a random effect. Bold type indicates significant effects ($P < 0.05$). The analyses were run for principal components obtained in the PCA based on fish behavioural variables (Table A2). RM: repeated measures effect.

Table A7
Cox proportional hazards model to test the latency to move after the introduction to the open field tank shown by the European bullhead and racer goby depending on the time of day

Variable	Effect	*df*	χ^2	*P*
Latency to the first movement	Species	1	16.07	**<0.001**
	Time of day	1	2.31	0.129
Latency to the first SZ -> DZ transition	Species	1	3.95	**0.047**
	Time of day	1	0.32	0.571

SZ: Safe Zone; DZ: Dangerous Zone. Bold type indicates significant effects ($P < 0.05$).

Table A8
Two-way general linear models to test the shelter occupancy of the gudgeon and monkey goby depending on the time of day

Principal component	Effect	*df*	Mean square	*F*	*P*
PC1	Species	1	7.39	8.89	**0.004**
Shyness	Time of day	1	4.92	5.92	**0.018**
	Residuals	61	0.83		
PC2	Species	1	0.14	0.13	0.717
Fear	Time of day	1	0.05	0.05	0.828
	Residuals	61	1.03		
PC3	Species	1	0.14	0.14	0.709
Time outside the shelter	Time of day	1	4.46	4.65	**0.035**
	Residuals	61	0.96		

The Species and Time of day were set as between-subject factors. Bold type indicates significant effects ($P < 0.05$). The analyses were run for principal components obtained in the PCA based on fish behavioural variables (Table A3).

Table A9
Three-way general linear models to test the response of the gudgeon and monkey goby to the novel object depending on the time of day

Principal component	Effect	*df*	Mean square	*F*	*P*
PC1	Species (S)	1	8.33	10.27	**0.002**
Activity	Time of day (D)	1	5.64	6.95	**0.010**
	Treatment (T)	1	2.24	2.75	0.100
	S*T	1	4.93	6.08	**0.016**
	Residuals	91	0.81		
PC2	Species (S)	1	3.29	7.62	**0.007**
Object avoidance	Time of day (D)	1	4.27	9.90	**0.002**
	Treatment (T)	1	37.38	86.69	**<0.001**
	S*D	1	1.44	3.34	0.071
	S*T	1	2.28	5.29	**0.024**
	D*T	1	7.97	18.49	**<0.001**
	Residuals	89	0.43		
PC3	Species (S)	1	1.55	2.51	0.117
Low number of object inspections	Time of day (D)	1	3.53	5.72	**0.019**
	Treatment (T)	1	16.19	26.21	**<0.001**
	S*D	1	4.04	6.54	**0.012**
	S*T	1	3.62	5.86	**0.018**
	D*T	1	11.11	17.98	**<0.001**
	Residuals	89	0.62		
PC4	Species (S)	1	0.01	0.01	0.915
Fear	Time of day (D)	1	0.59	0.78	0.380
	Treatment (T)	1	0.11	0.14	0.708
	S*D	1	3.69	4.91	**0.029**
	S*T	1	3.20	4.27	**0.042**
	D*T	1	20.57	27.39	**<0.001**

The Species, Time of day and Treatment (novel object present or absent) were set as between-subject effects. Bold type indicates significant effects ($P < 0.05$). The analyses were run for principal components obtained in the PCA based on fish behavioural variables (Table A3).

Table A10
Three-way general linear model to test the behaviour of the gudgeon and monkey goby in the open field depending on the Time of day and two periods

Principal component	Effect	*df*	Mean square	*F*	*P*
PC1	Species (S)	1	13.75	32.99	**<0.001**
Inactivity	Time of day (D)	1	5.64	13.52	**0.001**
	Period^{RM} (P)	1	3.80	9.12	**0.004**
	S*D	1	0.00	0.00	0.982
	S*P^{RM}	1	10.80	25.91	**<0.001**
	D*P^{RM}	1	3.07	7.37	**0.009**
	S*D*P^{RM}	1	4.30	10.32	**0.002**
PC2	Species (S)	1	0.21	0.41	0.526
Explorative behaviour	Period^{RM} (P)	1	4.71	9.06	**0.004**
	S*P^{RM}	1	4.89	9.41	**0.003**
PC3	Species (S)	1	2.42	5.16	**0.027**
Boldness	Time of day (D)	1	0.83	1.78	0.187
	Period^{RM} (P)	1	4.00	8.53	**0.005**
	S*D	1	0.54	1.15	0.289
	S*P^{RM}	1	2.53	5.38	**0.024**
	D*P^{RM}	1	1.54	3.29	0.075
	S*D*P^{RM}	1	2.82	6.00	**0.017**

Species and Time of day were set as between-subject fixed effects, Period (early response, immediately after the introduction to the open field tank, and late response, after the initial acclimation) as a within-subject (repeated measures) fixed effect and fish ID as a random effect. Bold type indicates significant effects ($P < 0.05$). The analyses were run for principal components obtained in the PCA based on fish behavioural variables (Table A3). RM: repeated measures effect.

Table A11
Cox proportional hazards model to test the latency to move after the introduction to the open field tank shown by the gudgeon and monkey goby depending on the time of day

Variable	Effect	*df*	χ^2	*P*
Latency to the first movement	Species	1	8.74	**0.003**
	Time of day	1	1.65	0.199
Latency to the first SZ -> DZ transition	Species	1	0.50	0.481
	Time of day	1	1.57	0.210

SZ: Safe Zone; DZ: Dangerous Zone. Bold type indicates significant effects ($P < 0.05$).

Augustyniak et al. (2024) - Supplementary material (Shelter occupancy test)

Table represents descriptive statistics for behaviours measured in the shelter occupancy test for all tested species - Shelter occupancy test.

	European bullhead				Racer goby				Gudgeon				Monkey goby			
Species	Day		Night		Day		Night		Day		Night		Day		Night	
Time of the day	20		20		16		16		16		16		16		16	
N	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Statistic	89.66	24.25	66.54	34.99	78.5	25.39	59.26	35.02	36.32	39.44	29.16	33.58	47.86	42.2	16.59	23.44
Time in the shelter	3.89	11.02	11.6	10.62	11.25	11.85	9.08	6.51	2.86	4.76	4.35	7.06	4.31	4.74	6.85	13.15
Time in the IZ	1425.05	645.43	798.79	701.51	835.72	621.18	721.2	624.62	886.15	866.56	443.56	598.1	353.86	494.44	164.96	241.19
Latency to the first IZ visit	0.84	2.6	3.39	4.11	4.8	7.49	7.96	7.07	5.27	7.69	5.14	4.42	6.16	4	8.46	7.59
Time in the NSZ	1080.84	903.67	363.39	736.98	342.3	723.26	345.46	721.68	1007.56	852.35	489.61	613.96	322.99	486.57	155.92	219.08
Latency to the first NSZ visit	4.39	12.77	15.26	19.51	5.45	9.56	22.02	23.67	10.82	19.19	45.38	37.51	32.03	31.56	56.97	27.13
Time in the DZ	1545.5	621.44	1118.43	758.17	1404.52	547.01	1012.98	711.48	1106.17	847.47	546.86	685.27	421.72	593.32	316.57	472.82
Latency to the first DZ visit	2.11	4.33	4.79	4.68	2.32	2.54	5.06	4.41	3.68	4.7	9.03	5.52	8.96	6.47	12.08	4.39
Distance to the shelter entry	2.45	4.54	9.2	8.73	7	7.1	7	5.15	5.19	7.59	4.5	4.38	4.31	4.08	2.63	3.36
Number of the shelter exits	2.3	7.12	12.85	18.53	8.63	17.43	11.06	11.22	8.75	12.24	7.75	6.89	9.06	9.13	8.31	8.02
Number of the NSZ visits	4.35	15.81	14.5	19.94	3.75	7.64	12.31	13.72	7.56	11.08	17.5	17.58	18.25	26.57	33.31	20.09
Number of the DZ visits																

Description:	Time spent in the shelter, expressed as a % of the total experiment time
Time in the shelter	Time spent in Inspect Zone, expressed as a % of the total experiment time
Time in the IZ	Latency to the first visit in Inspect Zone, expressed in seconds (maximum value of 1800 means that the fish did not visit this zone)
Latency to the first IZ visit	Time spent in Near Shelter Zone, expressed as a % of the total experiment time
Time in the NSZ	Latency to the first visit in Near Shelter Zone, expressed in seconds (maximum value of 1800 means that the fish did not visit this zone)
Latency to the first NSZ visit	Time spent in Dangerous Zone, expressed as a % of the total experiment time
Time in the DZ	Latency to the first visit in Dangerous Zone, expressed in seconds (maximum value of 1800 means that the fish did not visit this zone)
Latency to the first DZ visit	Mean distance to the shelter enty, expressed in cm
Distance to the shelter entry	Number of the shelter exits
Number of the shelter exits	Number of Near Shelter Zone visits
Number of the NSZ visits	Number of Dangerous Zone visits
Number of the DZ visits	

Augustyniak et al. (2024) - Supplementary material (Novel object test)

Table represent descriptive statistics for behaviours measured in the novel object test for all tested species - Novel object test.

Species	European bullhead								Racer goby							
Time of day	Day				Night				Day				Night			
Treatment	Control		Treatment		Control		Treatment		Control		Treatment		Control		Treatment	
N	12		16		12		12		10		10		10		10	
Statistic	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Time in the SZ	91.19	11.89	37.47	29.66	69.02	21.83	57.53	45.09	62.62	43.63	30.92	24.88	72.46	12.41	40.78	32.39
Time in the IZ	0.08	0.17	53.59	28.80	0.62	0.88	35.18	41.85	1.08	1.64	51.18	29.48	4.54	2.87	51.31	32.24
Latency to the first IZ visit	919.70	472.52	213.74	408.15	762.10	461.99	569.35	555.83	680.82	552.41	42.14	43.62	69.44	76.22	151.22	167.77
Time in the OZ	0.01	0.03	3.91	9.29	0.11	0.24	7.07	18.93	0.09	0.18	13.12	18.90	2.04	2.29	5.76	10.19
Latency to the first OZ visit	1079.72	340.08	602.51	468.08	996.21	341.36	1028.55	384.10	951.18	451.79	557.60	437.14	109.78	92.18	657.62	502.28
Distance to the OZ	16.74	1.28	10.15	3.69	16.45	0.72	11.93	5.73	16.20	0.81	9.26	3.73	15.10	0.81	9.45	3.50
Latency to the first movement	148.43	344.74	369.10	372.68	22.82	57.55	1060.13	265.66	39.30	66.55	115.68	126.51	3.54	4.66	520.62	359.43
Time on the move	23.58	19.45	30.76	24.87	58.59	27.58	3.11	8.06	43.56	22.01	28.79	21.27	57.11	13.77	15.37	17.37
Freezing event duration	208.02	286.66	98.37	122.46	22.96	33.83	141.81	259.57	43.61	18.72	34.76	24.51	32.62	18.01	57.27	52.65
Number of the IZ visits	0.75	1.48	24.56	22.99	3.17	5.18	6.25	12.73	4.50	6.13	23.20	20.31	23.40	10.52	10.20	6.68
Number of the OZ visits	0.17	0.39	0.00	0.00	1.00	1.86	0.00	0.00	1.20	1.99	0.00	0.00	8.50	4.40	0.00	0.00
Freezing events number	4.75	2.34	2.00	1.67	1.58	2.54	1.25	1.06	4.60	4.43	3.30	3.23	3.10	2.64	3.40	3.41

Species	Gudgeon								Monkey goby							
Time of day	Day				Night				Day				Night			
Treatment	Control		Treatment		Control		Treatment		Control		Treatment		Control		Treatment	
N	12		12		12		12		12		12		12		12	
Statistic	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Time in the SZ	74.71	29.81	21.87	29.21	55.42	18.90	77.93	20.22	84.10	17.85	51.56	25.65	43.69	12.20	70.85	15.58
Time in the IZ	3.95	6.36	64.20	35.82	4.37	7.43	18.44	18.07	0.02	0.03	11.12	24.56	3.83	5.97	0.35	0.38
Latency to the first IZ visit	558.87	508.77	276.40	361.35	231.77	212.06	724.04	431.23	951.70	389.45	502.05	435.40	79.73	97.74	511.15	395.53
Time in the OZ	0.55	0.93	3.36	6.74	0.47	0.39	0.45	1.25	0.01	0.02	6.62	16.72	1.20	1.76	0.10	0.18
Latency to the first OZ visit	667.52	499.56	534.15	510.51	391.07	343.63	979.28	381.89	1033.23	318.77	1018.08	408.06	260.59	413.21	1200.00	0.00
Distance to the OZ	15.63	1.98	7.24	3.88	16.57	1.65	14.78	2.81	16.57	1.11	14.07	5.49	16.50	1.16	16.82	0.88
Latency to the first movement	312.85	401.96	358.68	426.88	28.18	66.32	481.33	468.51	202.30	381.54	218.38	330.75	2.40	5.27	131.37	340.74
Time on the move	36.28	36.50	9.28	19.44	85.10	18.94	23.63	26.74	33.13	28.30	50.51	33.90	75.68	14.50	61.29	25.53
Freezing event duration	179.24	340.08	409.72	487.60	19.60	39.42	209.27	347.46	56.95	46.77	132.43	337.81	21.18	23.27	29.67	23.61
Number of the IZ visits	8.83	11.02	15.58	18.30	20.75	24.44	12.92	20.83	0.67	0.98	6.58	6.22	33.83	38.05	5.42	7.42
Number of the OZ visits	2.25	2.96	0.00	0.00	4.33	3.60	0.08	0.29	0.25	0.45	0.00	0.00	11.75	14.12	0.00	0.00
Freezing events number	2.75	2.45	2.42	1.88	1.17	1.95	4.92	2.97	4.58	3.99	1.58	1.83	1.00	1.48	2.17	2.17

Description:

Time in the SZ	Time spent in Safe Zone, expressed as a % of the total experiment time
Time in the IZ	Time spent in Inspect Zone, expressed as a % of the total experiment time
Latency to the first IZ visit	Latency to the first visit in Inspect Zone, expressed in seconds (maximum value of 1200 means that the fish did not visit this zone)
Time in the OZ	Time spent in Object Zone, expressed as a % of the total experiment time
Latency to the first OZ visit	Latency to the first visit in Object Zone, expressed in seconds (maximum value of 1200 means that the fish did not visit this zone)
Distance to the OZ	Mean distance to the Object, expressed in cm
Latency to the first movement	Latency to the first movement, expressed in seconds (maximum value of 1200 means that the fish did not start the acivity)
Time on the move	Time spent on the move, expressed as a % of the total experiment time
Freezing event duration	Mean duration of freezing event (> 30s without movement), expressed in seconds
Number of the IZ visits	Number of Inspect Zone visits
Number of the OZ visits	Number of Object Zone visits
Freezing events number	Number of freezing events (>30 s without movement)

Augustyniak et al. (2024) - Supplementary material (Open field test)

Table represents descriptive statistics for behaviours measured in the open field test for all tested species - Open field test.

Species	European bullhead								Racer goby							
Time of day	Day				Night				Day				Night			
Period	Early response		Late response		Early response		Late response		Early response		Late response		Early response		Late response	
N	16		16		16		16		16		16		16		16	
Statistic	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Time in the DZ	5.55	7.20	0.67	1.21	20.19	23.54	8.79	9.98	64.85	39.72	15.87	14.99	47.76	45.64	23.47	10.49
Time on the move	62.67	29.41	15.29	17.89	37.71	24.22	18.88	14.16	4.19	9.47	25.63	23.83	3.46	8.65	40.18	14.29
Freezing event duration	113.39	180.95	349.00	386.25	102.87	128.96	68.01	143.38	460.59	518.44	119.14	157.05	667.72	545.79	22.23	20.48
Unique segments in the SZ	7.69	1.25	5.63	2.87	7.19	2.07	7.44	1.75	2.00	2.92	6.63	2.33	2.44	2.90	7.94	0.25
Unique segments in the DZ	13.31	7.56	4.19	6.30	16.19	7.36	10.31	7.97	5.88	7.08	14.06	8.43	5.63	7.80	20.56	4.72
Number of the DZ visits	0.75	1.06	0.13	0.34	1.25	1.29	2.00	4.34	1.38	2.60	5.94	7.67	1.44	2.99	7.25	8.43
Freezing events number	1.25	1.61	3.13	1.96	1.13	1.31	3.38	3.30	3.81	2.97	5.19	3.78	3.13	2.87	2.50	3.69
Latency to the first SZ to DZ transition	885.00	448.31	-	-	879.73	380.01	-	-	1073.35	209.02	-	-	1103.24	188.64	-	-
Latency to the first movement	164.15	217.07	-	-	448.55	430.14	-	-	852.78	390.09	-	-	666.52	533.17	-	-

Species	Gudgeon								Monkey goby							
Time of day	Day				Night				Day				Night			
Period	Early response		Late response		Early response		Late response		Early response		Late response		Early response		Late response	
N	16		16		16		16		16		16		16		16	
Statistic	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Time in the DZ	32.01	25.58	53.25	33.60	53.86	37.14	26.48	32.11	15.28	8.38	3.74	4.40	22.66	23.89	10.41	22.03
Time on the move	31.76	31.83	44.46	32.17	37.50	37.29	81.28	27.78	65.61	34.54	43.02	31.47	88.54	8.64	61.70	27.93
Freezing event duration	127.58	133.40	218.20	345.19	172.39	294.60	7.49	16.28	45.72	62.78	95.44	296.42	8.83	15.85	12.24	18.77
Unique segments in the SZ	7.50	1.51	7.25	2.02	6.13	2.55	6.75	1.91	7.88	0.50	7.38	1.63	6.88	2.53	6.37	2.55
Unique segments in the DZ	17.31	9.02	20.38	6.03	18.06	6.19	16.50	9.03	20.38	5.38	8.69	8.60	19.69	8.81	10.50	7.31
Number of the DZ visits	13.31	14.37	10.50	9.66	8.00	9.29	17.87	38.61	31.69	24.29	16.19	28.78	43.63	47.21	35.13	81.10
Freezing events number	4.94	3.86	2.75	2.44	5.37	3.81	0.25	0.58	1.87	2.28	1.44	2.87	0.31	0.60	0.75	1.24
Latency to the first SZ to DZ transition	478.63	417.69	-	-	385.41	373.84	-	-	300.45	311.09	-	-	244.15	386.14	-	-
Latency to the first movement	157.12	337.64	-	-	137.76	187.09	-	-	116.35	225.22	-	-	31.01	71.60	-	-

Description:

Time in the DZ	Time spent in Dangerous Zone, expressed as a % of the total experiment time
Time on the move	Time spent on the move, expressed as a % of the total experiment time
Freezing event duration	Mean duration of freezing event (> 30s without movement) expressed in seconds
Unique segments in the SZ	Unique segments visited in Safe Zone, from 0 to 8
Unique segments in the DZ	Unique segments visited in Safe Zone, from 0 to 24
Number of the DZ visits	Number of Dangerous Zone visits
Freezing events number	Number of freezing events (>30 s without movement)
Latency to the first SZ to DZ transition	Latency to the first trasition from Safe to Dangerous Zone, expressed in seconds (maximum value of 1200 means that the fish did not made the transition)
Latency to the first movement	Latency to the first movement, expressed in seconds (maximum value of 1200 means that the fish did not start the acivity)

Manuskrypt

Augustyniak M, Kobak J, Jermacz Ł, Adamiak A, Kakareko T. Public information use – are invasive demersal fish species more effective than natives?

Manuskrypt przygotowany do wysłania do czasopisma

Public information use – are invasive demersal fish species more effective than natives?

Abbreviated title: Public information use by demersal invasive vs native fish

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Conflict of interest

The authors declare no competing interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Mateusz Augustyniak, upon request.

Author contributions

Conceptualization: Mateusz Augustyniak, Jarosław Kobak, Tomasz Kakareko; Methodology: Mateusz Augustyniak, Jarosław Kobak, Tomasz Kakareko; Formal analysis and investigation: Mateusz Augustyniak, Jarosław Kobak, Łukasz Jermacz, Agnieszka Adamiak; Writing - original draft preparation:

Mateusz Augustyniak; Writing – Review & Editing: Jarosław Kobak, Łukasz Jermacz, Agnieszka Adamiak, Tomasz Kakareko; Funding acquisition: Tomasz Kakareko; Resources: Mateusz Augustyniak, Łukasz Jermacz, Tomasz Kakareko; Supervision: Tomasz Kakareko

Lay Summary

The ability of invasive bottom-dwelling fish to use public cues released by natives can be linked with the spread of the former in new areas. Fish can use public cues, presented inadvertently by individuals of their own or other species, to recognize danger and food location. We proved experimentally that invasive bottom-dwelling fish can use public cues more efficiently (from a wider range of species) than sympatric native species, which may contribute to the invasion success of the former.

Abstract

Organisms determine environmental quality using their senses and personal experience (personal information), but can also use by-products of other individuals' activities, i.e. public information. The ability to use public information originating from both con- and heterospecifics gives an advantage over individuals relying only on personal information or conspecific cues. The role of public information in invasion ecology is of high concern, as any differences in this aspect between alien and native species may determine the success of the former. Here we used two pairs of sympatric invasive and native demersal fish species (racer goby *Babka gymnotrachelus* / European bullhead *Cottus gobio*; monkey goby *Neogobius fluviatilis* / gudgeon *Gobio gobio*) facing two types of public cues (associated with frightened and foraging individuals) as a model to check if the invaders are more effective in public information use than the natives. Both invaders and the native gudgeon used danger cues from con- and heterospecifics, while the native bullhead failed to recognize heterospecific danger cues. The monkey goby and both native species appear to be attracted to foraging cues from donors less likely to exert competitive pressure on the observer (i.e. native species rather than potentially more aggressive invaders), while the racer goby appeared unable to correctly recognize heterospecific cues. Our results showed that public cues can enable invaders to read threat from a wide range of individuals and to find optimal food patches, which may contribute to their invasion success.

Keywords: biological invasions, cognitive abilities, food location, goby, predation risk, social information

Invasive species constitute a great threat to global biodiversity (Rodriguez 2006; Dueñas et al. 2021). Freshwater ecosystems, which cover only around 0.8 % of the Earth's surface, but provide habitats for around one-third of all vertebrate species, are particularly vulnerable to biodiversity loss (Dudgeon et al. 2006). They are subject to high anthropogenic pressure, including habitat modifications which make them less suitable habitats for native organisms. However, invasive, usually opportunistic species may benefit from human impact and successfully establish themselves in human-modified environments (e.g. Koehn 2004; Scott 2006; Johnson et al. 2008). Invasive species appear in freshwater ecosystems because of human activities, such as aquaculture, transport by ships, commercial stocking and recreational activities (Nunes et al. 2015). Thus, fresh waters are recipients of a huge number of invasive species, especially fish, which belong to the most commonly introduced animals in the world (Gozlan 2008; Bernery et al. 2022).

Invasive species often differ substantially from natives and usually their appearance in novel habitats causes consequences for native communities (Ricciardi and MacIsaac 2011). The establishment of an invasive species leads to changes in local communities, in which the invader interacts with the natives (especially those phylogenetically related or ecologically similar), forming new relationships and modifying existing ones. These interactions may be direct or indirect and include predation, hybridization, disease transmission and/or competition (Genovesi et al. 2015). Invasive fish species usually exhibit a generalist diet, broad environmental tolerance, high phenotypic plasticity and high reproductive success (Bernery et al. 2022), which provide them with a competitive advantage over native organisms. However, some aspects of the ecology of invasive fish and their consequences for native aquatic communities are still poorly understood, one of them being the ability to use and/or provide public information by invaders.

Organisms collect information about their environment by direct interactions with its elements, using their senses ("personal information use") and personal experience from the past. In addition, they can use social information, provided by other individuals (Danchin et al. 2004). One of the social information types is public information, based on inadvertent cues being by-products of other individuals' activities, such as behaviour (Bonnie and Earley 2007; Valone 2007). The ability to use the knowledge of other individuals gives an advantage over those relying only on their personal information. The public information cues may originate from conspecifics or heterospecific individuals (Danchin et al. 2004), particularly within the same ecological guild, coexisting for a long time. Using these cues may be particularly useful in novel environments (Terkel 1995), as individuals need to collect information about predation risk and available resources as soon as possible to increase their chances of successful establishment. Thus, a special case of the role of public information use in ecosystem functioning is the establishment of invasive species, entering an unknown community and being potential users and demonstrators (donors) of cues within that community. Any differences in this aspect between the alien and native species may contribute to the success or failure of the former in a novel location.

A good example of invasive species that have joined and altered freshwater fish communities on a large scale is that of the goby fish (Gobiidae) originating from the Ponto-Caspian region. Several species of goby spread spectacularly in Europe (Copp et al. 2005; Roche et al. 2013; Grabowska et al. 2023) and the Laurentian Great Lakes of North America (Kornis et al. 2012). They constitute a specific group of solitary (i.e. not showing schooling behavior), bottom-dwelling, shelter-associated species that may cause biocoenotic changes, particularly in local fish communities. Indeed, there is evidence that invasions of the gobies displace or threaten native demersal fish species (Kornis et al. 2012; van Kessel et al. 2016) by outcompeting them for food (Kakareko et al. 2013) or shelter (Jermacz et al. 2015; Błońska et al. 2016). In European fresh waters, missing native gobies, these Ponto-Caspian invaders interact with phylogenetically distant but ecologically similar fish species. However, the relationships between these species in the context of public information use remain unclear. It was not known whether and how the invaders participate in public information use in such new fish communities. If so, are they superior to their native analogous species in this respect? Can they also act as public information demonstrators for native species?

Our main goal was to check differences in the public information use between co-occurring gobies and native fish species. We performed a series of experiments to check if the fish can use public information cues from con- and heterospecifics. We tested two types of cues differing in their immediate importance for the survival of the tested individual. In the first scenario, public information demonstrators presented a fright reaction. This was considered a “strong” stimulus, as failure to use such information properly may put an individual in a life-threatening situation. In the second scenario, test fish were confronted with public information produced by foraging individuals. This was considered a “weak” stimulus, as, if the fish are not able to use such information properly, they only risk staying hungry.

We hypothesized that the invasive species would use public information more effectively than natives. Specifically, invaders will change their behavior in response to public information cues provided by both conspecifics and heterospecifics irrespective of the stimulus type. In turn, we assumed that the native species would recognize cues provided by con- and heterospecifics displaying a strong stimulus but only by conspecifics exhibiting weaker cues. This is because, at the beginning of the invasion process, conspecifics are unfamiliar with the new environment and present in low numbers (Terkel 1995; Camacho-Cervantes et al. 2015) and the ability to recognize diverse cues, including those from other species, may be beneficial (Hazlett 2000; Damas-Moreira et al. 2018) for the invader. Thus, individuals with such an ability would exhibit higher fitness and contribute to the future invasive population to a greater extent. Also, enhanced cognitive abilities might help them outcompete their native competitors (Szabo et al. 2020). Clarifying this issue would allow a better understanding of mechanisms that may contribute to the successful dispersal of invasive species in new environments.

MATERIALS AND METHODS

Animals

We tested the Ponto-Caspian gobies and their native analogues arranged in two pairs consisting of a co-existing invasive vs. native species. We used the racer (*Babka gymnotrachelus,* (Kessler 1857)) and monkey goby (*Neogobius fluviatilis*, (Pallas 1814)), both invasive in European fresh waters (Copp et al. 2005; Grabowska et al. 2023). We chose the European bullhead (*Cottus gobio* (Linnaeus 1758)), as a native counterpart for the racer goby, as their ranges overlap and they are known to co-exist in the same habitats (Kakareko et al. 2016; Janáč et al. 2018). Both these species are strongly associated with the bottom and shelters. For a native counterpart of the monkey goby, we selected the gudgeon (*Gobio gobio* (Linnaeus 1758)). Both these species occupy sandy bottom areas (Kottelat and Freyhof 2007; Płąchocki et al. 2020) and are likely to interact with each other. Jakovlić et al. (2015) observed a decline in a gudgeon population when the monkey goby density increased, which suggests the competition between species in the wild.

Fish were collected in the autumn of 2021. European bullhead and racer goby were collected from the Brda River (53°08'52.5"N 17°58'10.5"E), by a diver using an aquarium net. Gudgeon and monkey goby were collected from the Pilica River (51°45'49.0"N 21°08'56.7"E) by electrofishing (EFGI 650, BSE Bretschneider Spezialelektronik, Germany). The fish were transported (2-3h) in sealed plastic bags filled with oxygenated water, and placed inside a Styrofoam cooler to maintain a constant temperature. After transport, they were kept in an air-conditioned stocking room in single-species 420-l stock tanks (max. 50 individuals per tank). The tanks were filled with conditioned tap water (mean temperature ± SD: 16.5 ± 0.5 °C, pH: 8.1 ± 0.2, electrical conductivity: 603 ± 4 µS/cm, oxygen level: 8.1 ± 0.4 mg/l, 82.2 ± 3.7 %; measured with a Multi 340i Meter, WTW, Weilheim, Germany). The stock tanks were equipped with aquarium filters and aerators. Ceramic and stony shelters were provided, but no bottom substrate. The photoperiod was set at 12:12 h L:D cycle, with lights on at 0700. The fish were fed daily *ad libitum* with frozen chironomid larvae. The water was exchanged once a week (c.a. 30% of the water volume). The filters were cleaned during each water exchange.

Two weeks before the start of the experiments, the fish were moved to pre-experimental tanks (60 x 40 x 35 cm; length x width x height; water level: 30 cm), devoid of any shelters and bottom substrate (10 individuals per tank). They were filled with the same conditioned tap water as experimental tanks. This was to make the fish more familiar with the lack of shelters, which were absent in the experimental tank as well. Fish of each species were kept separately and there was no visual contact between individuals of different species. The maintenance of the pre-experimental tanks was the same as that of the stock tanks (feeding, water exchange, filter cleaning).

The total length (TL) of the fish was measured using the video frames of experimental recordings and the ImageJ (Schneider et al. 2012) program (see Results section).

Ethical note

The present study adheres to the ASAB/ABS guidelines for the use of animals in research (ASAB Ethical Committee & ABS Animal Care Committee, 2020). The fish were collected and used under the permit of the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland, statement no. 51/2021. The capture and use of the European bullhead, partially protected by law in Poland, was approved by the Regional Directorate for Environmental Protection in Poland (approval numbers: WOP.6401.4.5.2017.MO, WOP.6401.4.19.2018.MO, WOP.6401.4.52.2022.MO).

Fish manifested overall activity and food intake, which confirmed that housing conditions guaranteed good animal welfare throughout the research period. We did not observe any external signs of stress or disease (e.g., unnatural body shape, skin changes, swimming problems). After the experiments, the European bullhead and gudgeon were released where they had been caught. Invasive gobies were euthanized by an overdose of Tricaine Methanesulfonate (MS-222).

The main idea of the experiment

Our study aimed to investigate the ability of invasive fish to use public information. We were interested in how the invaders integrate into freshwater communities in terms of exchanging (i.e. using and providing) information with native heterospecifics. In the first experiment, we checked if fish can recognize danger based on public information cues from con- and heterospecifics within each pair. If so, we expected the test fish to avoid the area near the cue demonstrators and reduce their activity, as these are well-known anti-predator behaviors (Lima and Dill 1990; Teplitsky and Laurila 2007; Kłosiński et al. 2022; Augustyniak et al. 2023). In the second experiment, we checked if fish are able to differentiate between foraging and non-foraging demonstrators of the public information cues. If so, we expected them to approach the foraging demonstrators. In both experiments, we confronted fish with conspecific and heterospecific cues within each pair. Additionally, in the second experiment, we confronted the fish from the first pair (racer goby and European bullhead) with heterospecific cues from the gudgeon, a native species commonly present in rivers, also in the Brda River, from which the racer goby and European bullhead were collected (Radtke et al. 2015). This allowed us to check whether the native fish can recognize and discriminate between heterospecific cues released by two heterospecifics: one invasive and the other native. Also, we checked whether the invasive species reacted differently to the cues from different natives. The cue demonstrators were not used later in the experiment.

Experimental setup

We used LCD computer monitors to present the cues to the fish tested. To make sure that the fish were able to see and recognize the cues shown on the monitors, we performed a preliminary test. We recorded a short video showing the introduction of food, viz. frozen chironomids, to the tank. The food was applied with a pipette, in the same way the fish were fed on a daily basis. Then, we displayed this video on the monitors to the fish in the stock tank (using the same monitors as in the actual experiments, facing the stock tank wall). All the test species reacted to the displayed chironomids by approaching the monitor and swimming up to the surface to grab the food.

The experimental setup consisted of a glass experimental tank (30 x 30 x 30 cm; length x width x height), with two computer monitors (BenQ GW2280-B, Taipei) placed on two opposite sides of the tank (Figure 1) to display the stimulus videos (1 cm from the tank wall). The whole tank (except the areas where the stimuli were displayed on the monitors) was covered by Styrofoam screens. The experimental tank was filled with conditioned (24-h aged and aerated) tap water (mean temperature ± SD: 16.9 ± 0.9 ºC, pH: 8.6 ± 0.1, conductivity: 661 ± 25 µS/cm, oxygen level: 8.5 ± 0.4 mg/l, 87.6 ± 4.0 %;). The water level in the tank was 5 cm, which was sufficient for test fish to exhibit natural behavior based on preliminary tests, and allowed to limit their vertical movements, potentially interrupting our observations from the top. An IP camera (Samsung SNB-6004P, Changwon, South Korea) was placed 50 cm above the water surface to record the fish behavior. The whole setup (the experimental tank and monitors) was covered by white Styrofoam screens to prevent the fish from receiving any external stimuli. A Styrofoam screen was also placed below the tank to provide a white homogenous background for better fish visibility on recorded videos.

Stimulus preparation

Each stimulus video (displaying the foraging or fright reaction of demonstrator fish of a particular species) consisted of a sequence of videos described below. Videos to be used as a stimulus (1920 x 1080 pixels, 30 frames per second) were recorded in a rectangular demonstrator tank (30 x 20 x 15 cm; length x width x height) filled with 5 cm of conditioned tap water (the same as in the experimental tanks). The camera recording the cues faced the longer wall of the demonstrator tank, which was of the same length as the wall of the experimental tank (30 cm). Therefore, the frame size of the stimulus videos matched the dimensions of the experimental tank and displayed demonstrators were shown in their actual size, corresponding to the size of the test individuals.

Experiment 1. Public information from individuals exhibiting a fright reaction

To prepare the stimulus for Experiment 1, three individuals of the same species (demonstrators) were placed in the demonstrator tank for 30 min acclimation. Then the experimenter presented a strong stressful stimulus using three circular (5 cm in diameter), red plastic objects suspended by a wire from a steel rod (Figure 2). The experimenter remained invisible to the fish, while red objects swinging above the tank caused a strong fright reaction of the demonstrators: an intensive period of erratic movements followed by a phase of immobility when the fish were squeezed into one corner of the tank.

Experiment 2. Public information from foraging individuals

To prepare the stimulus for Experiment 2, we recorded the behavior of foraging demonstrators. The demonstrator tank was equipped with two Tygon® tubes mounted on the longer wall of the tank and connected with a Watson-Marlow 323U (Watson-Marlow Fluid Technology Solutions, Falmouth, Cornwall, UK) peristaltic pump. The other end of each tube was placed in another tank of the same dimensions, containing a Petri dish with defrosted Chironomidae larvae used as a food source (Figure 3). One of the tubes was set up to provide the food to the demonstrator tank (to the “feeder” placed in the center of the tank wall), and the other tube was used to pump out excess water to maintain the water level at 5 cm during the recording (i.e. the tubes transported water in the opposite directions). This approach guaranteed that the food was provided in the same, repeatable manner, without any disturbance to the fish in the demonstrator tank. The IP camera was placed next to the demonstrator tank, 20 cm from the wall with the Tygon® tubes. There were two plasticine stripes on the outside surface of this wall. The vertical stripe covered the tube providing the food, and the horizontal stripe was put adjacent to the bottom of the tank. The stripes were used to make the food itself invisible (neither during the feeding phase nor later when lying on the bottom) in the videos, which was confirmed by watching the recorded videos. Thus, the only source of information about the food presence available to the fish tested in the experimental tank (observer) was the behavior of demonstrators, rather than the view of the food itself. The demonstrator tank was covered by white Styrofoam screens to keep the fish away from any external stimuli, while the second tank with food in a Petri dish remained uncovered, thus the experimenter had easy access to it without disturbing the demonstrators. First, we made a 5-min recording of an empty demonstrator tank (with no fish). Then we placed three individuals of the same species (food-deprived for 24 h) acting as demonstrators into the tank. The demonstrators had 30 min to acclimatize, during which the peristaltic pump was working (only water was pumped, but there was no food in the Petri dish). Then, the experimenter gently provided the first portion of food (5 defrosted Chironomidae larvae) to a Petri dish in the second tank. The food was sucked by the nearby tube into the tank with the demonstrators. Two min after the demonstrators consumed the first portion, the experimenter provided the next food portions (5 times in total, every 2 min).

Processing video recordings

For each experiment, we prepared two video sequences: the “stimulus sequence” and the “control sequence”. For Experiment 1, the “stimulus sequence” was a record of freely swimming demonstrators (30 min) followed by a record of intense erratic movements of the demonstrators (0.5 min) and a record of inactive demonstrators squeezed into one corner of the tank (30 s of this behavior multiplied to obtain a 4.5 min sequence). We showed intense erratic movements of the demonstrators for 30 s to reflect the short and intense nature of this behavior, which was observed during the cue recording. The “control sequence” contained a 35-min record of an empty demonstrator tank. Both sequences are presented in Figure 4a. We showed the stimulus video on only one monitor, as we were interested in the time spent near the stimulus monitor as a response variable. We used an empty tank, rather than freely swimming fish as a control sequence to be displayed on the other monitor, because a situation when frightened and freely swimming individuals are simultaneously present in the environment might be misleading.

In Experiment 2, the "stimulus sequence" was a record of an empty demonstrator tank (30 min) followed by a record of foraging demonstrators (10 min). To prepare the record of foraging demonstrators, a period of their most intense foraging was chosen (30 s since one of the food portions was added) and multiplied to obtain a 10 min long video of continuous foraging. The "control sequence" consisted of a record of an empty demonstrator tank (30 min) followed by a record of demonstrators swimming freely in the tank (without foraging, 10 min). Both sequences are presented in Figure 4b. We prepared one sequence of each type (stimulus and control) with each demonstrator species.

To minimize the disturbance of the test fish caused by a sudden change on the monitors, we have added smooth, slow transitions between particular video segments. One segment disappeared for 3 seconds, and simultaneously the second one started to appear. These effects were obtained using the video processing software Movavi Video Suite 18.

Experimental procedure

The experiments were conducted in May 2022, between 08:00 and 14:00 hours. The "stimulus" and "control" video sequences were switched between the monitors in each consecutive trial. In Experiment 1, fish from both pairs were divided into two groups facing con- or heterospecifics fright reaction. In Experiment 2, fish from the first pair (the racer goby vs European bullhead) were divided into three groups. The first group was exposed to the videos with the conspecific demonstrators, the second group was exposed to the videos with heterospecific demonstrators, i.e., individuals from the other species in each pair, and the third group was exposed to the videos with the gudgeon as demonstrators. The fish from the second pair (the monkey goby vs gudgeon) were divided into two groups and these were confronted with public information cues from con- or heterospecific (the other species from this pair) demonstrators, respectively. A single observer fish was placed in the experimental tank for 30 min acclimation period, during which both monitors were turned on and displayed the first part of the proper video (Figure 4). After 30 min, one monitor started to show the "stimulus" sequence, and the other one displayed the "control" sequence. The trial ended when both videos stopped (see Figure 4 for the duration of the sequences). The fish was netted from the experimental tank and transferred to the post-experimental tank. Each fish individual was used in only one trial of the particular experiment. We performed 10 trials for each species and variant (con- and heterospecific cues) of the experiment. The water in the experimental tank was totally exchanged after each trial.

In Experiment 1, we did not randomize the order of cue presentation to control for the effect of passing time. This is because the applied order is natural for a fish facing a danger. Thus, assuming that the test fish will recognize the presented cue, changing the order could be misleading.

Video analysis

To minimize observer bias, we used the Ethovision XT® 10.1 program to analyze the fish behavior in the videos recorded, with a sample rate of 5 frames per second. We were interested in fish behaviors in two periods: (1) the last 5 and 10 min of the acclimation period of Experiment 1 and 2, respectively, hereafter referred to as a pre-stimulus period (i.e. before presenting a stimulus), and (2) the period of the cue presentation. In Experiment 1, these were: 30 s of a fright reaction of demonstrators (hereafter: fright reaction period) and 4.5 min during which the demonstrators were inactive (hereafter: inactive demonstrator period). In Experiment 2, the cue was presented for 10 min (Figure 4), and hereafter we referred to this period as a stimulus period.

For Experiment 1, we set two zones on the experimental tank bottom, covering the whole tank width: (1) the “stimulus zone”, adjacent to the “stimulus” monitor (showing demonstrators) and (2) the “control zone”, adjacent to the monitor displaying the control sequence (Figure 5a). We measured the time spent by the test fish in each zone in each period (expressed as % of the total period time) and its general activity, expressed as the time spent on the move (% of the total period time).

To analyze Experiment 2, we set two zones on the experimental tank bottom: (1) the “foraging zone”, set up around the feeder on the monitor displaying foraging demonstrators, and (2) the “non-foraging zone” adjacent to the wall facing the monitor displaying the “control” sequence (Figure 5b). We measured the time spent by the tested fish in each zone (expressed as % of the total period time) in each period.

Statistical analyses

Fish total length was compared within the pairs using independent sample T-tests.

We calculated zone preference indices using times spent by fish in particular zones in both experiments, using the formula:

$$\frac{(\text{time spent in the zone of interest}) - (\text{time spent in the other zone})}{(\text{time spent in the zone of interest}) + (\text{time spent in the other zone})}$$

The value of "0" meant that the fish showed no preference for or avoidance of any zone. The positive and negative values meant that the fish showed a preference for or avoidance of the zone of interest, respectively.

To test the effects of observer species, demonstrator species and period on zone preference index (Experiments 1 and 2) and general fish activity (in Experiment 1), we performed Linear Mixed Models (LMM). The observer species and demonstrator species were set as between-subject fixed effects, the period as a within-subject fixed effect and the test fish ID as a random effect. In both experiments, we interpreted the main effect of period and its interactions with other factors as indicators of fish reaction to different types of cues presented. The parametric test assumptions were not violated based on the visual inspection of residual plots.

We performed model simplification by dropping the highest-order non-significant interaction terms and chose the best-fitting models based on the Akaike information criterion. We retained the more complex model when its AIC value was lower than that of the simpler model by 2 or more (Burham and Anderson 2002). For significant interactions, we performed LSD pairwise post-hoc comparisons with sequential Bonferroni corrected critical p-value. All analyses were performed were performed using IBM SPSS Statistics 29.0 (IBM Corp.).

RESULTS

The TL of the fish was (mean ± SD): 4.35 ± 0.51 cm for the European bullhead, 4.53 ± 0.52 cm for the racer goby, 5.37 ± 0.63 cm for the gudgeon, and 4.51 ± 0.61 cm for the monkey goby. The TL of the experimental fish did not differ between the European bullhead and racer goby (Experiment 1: $t_{58} = -1.25$, $p = 0.22$; Experiment 2: $t_{58} = -1.31$, $p = 0.20$). On the other hand, the gudgeon had greater TL than the monkey goby (Experiment 1: $t_{38} = 4.47$, $p < 0.01$; Experiment 2: $t_{38} = 5.01$, $p < 0.01$). As the fish in both pairs lived in sympatry and were randomly collected in the field, we considered that the size differences between the gudgeon and the monkey goby reflected those found in nature.

Responses of the European bullhead and racer goby

In Experiment 1 (fear cues), the LMM on the “stimulus zone” preference index showed a significant observer species x period interaction (Table 1). The European bullhead was less associated with the “stimulus zone” during the fright reaction period compared to the other periods regardless of the demonstrator species (Figure 6a). The LMM on activity showed a significant effect of observer species x demonstrator species x period interaction (Table 2). The European bullhead reduced its activity in the fright reaction period compared to the other two periods when demonstrators were conspecifics. The racer goby reduced its activity in the fright reaction period compared to the pre-stimulus period regardless of demonstrator species. When demonstrators were the European bullhead, the racer goby was less active in the inactive demonstrator period compared to the pre-stimulus period. In the pre-stimulus period, the racer goby was less active when facing freely swimming gobies compared to the racer goby viewing freely swimming bullhead. When demonstrator racer goby individuals presented a fright reaction, the observer racer goby were less active compared to the observer European bullhead (Figure 6b).

The LMM on the “foraging zone” preference index in Experiment 2 (foraging cues) showed a significant effect of an observer species x demonstrator species x period interaction (Table 3). The post-hoc analysis showed no significant differences between groups defined by these factors. However, there are visible tendencies that the European bullhead was more associated with the foraging zone when demonstrators were gudgeons and the racer goby exhibited the same behavior facing foraging racer gobies (Figure 7), which made this interaction significant.

Responses of the gudgeon and monkey goby

In Experiment 1 (fear cues), the LMM on the “stimulus zone” preference index showed a significant main effect of period (Table 4). The post-hoc tests showed no significant differences in preference index between different periods, however, the association of fish tended to be lower in the inactive demonstrators period compared to the other periods (Fig. 8A). For the activity, the LMM showed a significant effect of an observer species x period interaction (Table 5). The monkey goby reduced its activity in the fright reaction period compared to the pre-stimulus period regardless of the demonstrator species (Figure 8b).

In Experiment 2 (foraging cues), LMM indicated a significant effect of observer species x demonstrator species and demonstrator species x period interactions on the “foraging zone” preference index (Table 6). Both observer species were more associated with the "foraging zone" during the stimulus period compared to the pre-stimulus period when demonstrators were gudgeons (Figure 9).

DISCUSSION

The study aimed to check whether there are differences in the public information use between invasive gobies and sympatric native fish species in two scenarios differing in the level of cue importance for animal survival, i.e. public information about danger (strong stimulus) or feeding ground (weak stimulus). We have partially confirmed our hypothesis that invasive species use public information more effectively than natives in some contexts.

When confronted with a strong stimulus, both invasive species reduced their activity in response to cues from con- and heterospecifics, and the monkey goby tended to avoid inactive (after fright reaction) fish regardless of their species. Activity reduction and avoidance of dangerous areas are basic antipredator strategies (Lima and Dill 1990; Teplitsky and Laurila 2007), thus we can conclude that the invaders can effectively use this type of cue from individuals of the same and other species, which emphasizes their ability to avoid danger based on public information cues from a variety of species around.

Among the native species, only the European bullhead exhibited activity reduction and only in response to conspecifics. However, the bullhead avoided both con- and heterospecific frightened fish, and the gudgeon showed the same tendency in the presence of inactive demonstrators of both species. Considering both responses of the European bullhead to conspecifics, we can relate them to an antipredator response, where an endangered individual first tries to avoid a dangerous area and then reduces its activity to remain less conspicuous. Yet, when the fright behavior was demonstrated by the racer goby, the response of the European bullhead should be interpreted with care. Despite the avoidance of a dangerous area, the native species did not reduce its activity, which is in contrast with the results by Augustyniak et al. 2023, who showed low activity of the European bullhead facing a direct predator danger. We can interpret this lack of activity reduction in our current study in the light of the high aggressiveness of the racer goby towards the European bullhead individuals showed during shelter (Grabowska et al. 2016; Jermacz et al. 2015) and food competition (Kakareko et al. 2013). The native species, faced with the fright behavior of the racer goby, could interpret this as an attack attempt (i.e. did not interpret the cue properly), thus swimming away to avoid the risk of an attack, but stayed active and did not present the full repertoire of antipredator behaviors. The indicator of potential high aggressiveness of the racer goby towards the European bullhead was observed also in the current study when the racer goby was more active facing freely swimming bullheads than viewing other gobies. The study by Kakareko et al. (2016) showed that small (< 6 cm in TL) racer gobies can outcompete small European bullheads (also < 6 cm in TL) from habitats where both species coexist. As we used individuals of similar size in the current study, we may assume that the racer goby individuals tried to chase European bullheads away. The level of aggression of the racer goby towards conspecifics and European bullheads was found to be similar (Kakareko et al. 2013). Thus, it is possible that the racer goby facing other gobies were less active than in the presence of bullhead individuals to avoid a direct confrontation with conspecifics, associated with the high risk of injury. The native gudgeon showed a tendency to avoid inactive (after the fright reaction) con- and heterospecifics, but did not reduce its activity.

The behavior of gregarious gudgeon might differ when tested in conspecific groups. However, stressed gudgeon reduced its activity regardless if tested individually (Augustyniak et al. 2024) or in groups (Kłosiński et al. 2022). On the other hand, as the avoidance of dangerous areas seems to be the main antipredator behavior of the gudgeon facing a direct predator danger (Augustyniak et al. 2023), we may assume that it recognized public information cues from surrounding frightened individuals properly, being as effective as invasive gobies. To summarize, both invasive gobies as well as the native gudgeon effectively used cues about danger from con- and heterospecifics and exhibited antipredator response. However, behavioral responses of the native European bullhead were less clear (different behaviors facing con- and heterospecifics). We may assume that it is less effective in recognizing the nature of public information cues than the invaders, which in the natural environment may put the European bullhead in a life-threatening situation.

The fish confronted with the public information cues from foraging individuals exhibited a species-specific repertoire of behaviors, which can be explained by the theoretical competition-information quality trade-off (Gil et al. 2017). The concept assumes that the information (cue) is more valuable when the potential costs of competition with cue donors remain low, and these costs are greater when the distance between individuals (demonstrators and observers) in time, space and ecology is shorter (Seppänen et al. 2007). In the current study, the invasive monkey goby did not show any behavioral reaction to public information cues from foraging conspecifics. Following our hypothesis, we might expect that they will not be effective in using public information cues from a wide variety of species if they cannot use conspecific cues. However, in the light of the theoretical competition-information quality trade-off concept, this may be an adaptive behavior. Although conspecifics can provide more relevant public information than heterospecifics, the greater niche overlap between the conspecifics intensifies the competition for resources (Gil et al. 2017). Invasive gobies are known for their high intra- and interspecific aggressiveness (van Kessel et al. 2011; Kakareko et al. 2013; Grabowska et al. 2016). Thus, facing foraging conspecifics, the monkey goby may recognize the cue, but, because of the short distance between the demonstrators and observers in our study, they did not join an already occupied food patch avoiding a confrontation with aggressive conspecifics. Gil et al. (2017) showed that heterospecific groups are optimal in terms of foraging efficiency because of lower competition than in conspecific groups. Thus, the invader joined the food patch in response to gudgeon cues, as the risk of potential competition was lower than in the case of conspecifics, while the quality of information (i.e. potential food gains) still sufficient to trigger this behavior.

The behavior of the European bullhead in the current study also supports the theoretical competition-information quality trade-off concept, as this species, after assessing potential benefits (i.e. food gain) and costs (i.e. energy loss for competition), stayed away from foraging demonstrators of the same species. However, its reaction to public cues from foraging heterospecifics differed between demonstrator species. The European bullhead was attracted (though the tendency was non-significant) to foraging gudgeons. Both species coexist in the natural environment and, after recognizing a familiar representative of another species, the European bullhead joined the food patch since the potential risk of direct competition with the gudgeon was assessed as lower than that posed by the racer goby and conspecifics. The racer goby was able to outcompete the European bullhead in the laboratory study (Kakareko et al. 2013), and, accordingly, the native species did not join foraging gobies in the current study.

The invasive racer goby and native gudgeon, attracted to foraging conspecifics (though in the case of the racer goby this was a marginally significant tendency), seem to contradict the theoretical competition – information quality trade-off concept. The lack of reaction of the racer goby to foraging gudgeons can be accounted for by the habitat occupied by the racer goby. It is often found in stony areas (Kakareko et al. 2016), spending most of the time inside a shelter (Grabowska et al. 2016). It is possible that with such a specific lifestyle the racer goby does not often encounter pelagic species during foraging, and because of a short co-existence time, may not be able to assess properly the potential level of aggression posed by the gudgeon. Moreover, the racer goby was not attracted to foraging European bullhead. It is known that the racer goby can outcompete the European bullhead for food (Kakareko et al. 2013), so the potential competition cost was low for the invader. The possible reason why the racer goby did not join the foraging European bullhead may be that the invader did not recognize these public cues properly. Thus, it failed to assess the heterospecific information quality and to make a proper decision based on a potential cost-to-benefit trade-off. Gudgeon responses to foraging individuals can be considered concerning its gregarious nature, which makes it likely to rely more on conspecific information cues (Jones and Sieving 2019), and thus join foraging conspecifics. It could be that the gudgeon recognized public information cues from foraging monkey gobies, but, according to the theoretical competition-information quality trade-off concept, stayed away from foraging invaders to avoid direct competition. On the other hand, it is possible that both native species do not recognize properly public cues demonstrated by foraging invaders and avoid them instead of benefiting from their presence. This scenario suggests another facilitation mechanism for the invaders in new places.

The above considerations partially support our hypothesis about the greater effectiveness of public information use by invasive benthic fish compared to the natives. When there is an imminent danger associated with making the wrong decision based on public information cues, the invaders can use cues from con- and heterospecifics. This may be particularly helpful during the invasion process, as increasing the chance of survival when the invader encounters new enemies. The native gudgeon is able to use public danger cues from both con- and heterospecifics as effectively as the invasive gobies, while the native European bullhead turned out to be the least effective, and potentially most vulnerable to danger, presenting the full antipredator behavior only in response to frightened conspecifics. When public information cues are not associated with the immediate consequences for survival, all the test species can make a behavioral decision based on the cost and benefit trade-off assessment. In this case, proper reactions (attraction to foraging cues from fish posing a lower competitive pressure on the observer) were exhibited by the monkey goby and both native species, whereas the racer goby seemed not to be able to recognize properly heterospecific cues. Nevertheless, the ability of the invasive benthic fish to use public cues released by natives can be linked with the spread of the former into new habitats. Locally occurring species may provide them with public information about danger and resource locations, while the risk of direct competition with aggressive conspecifics will decrease (Grabowska et al. 2019). Moreover, the avoidance of foraging invasive species by natives observed in the current study may pose a risk of increasing dominance of invasive species, which will replace the native species from optimal food patches. Thus, as pointed out by Gil et al. (2017) and Seppänen et al. (2007), interspecific social information exchange may be an important factor shaping animal communities, which highlights the role of further research on the role of public information cues in invasion ecology.

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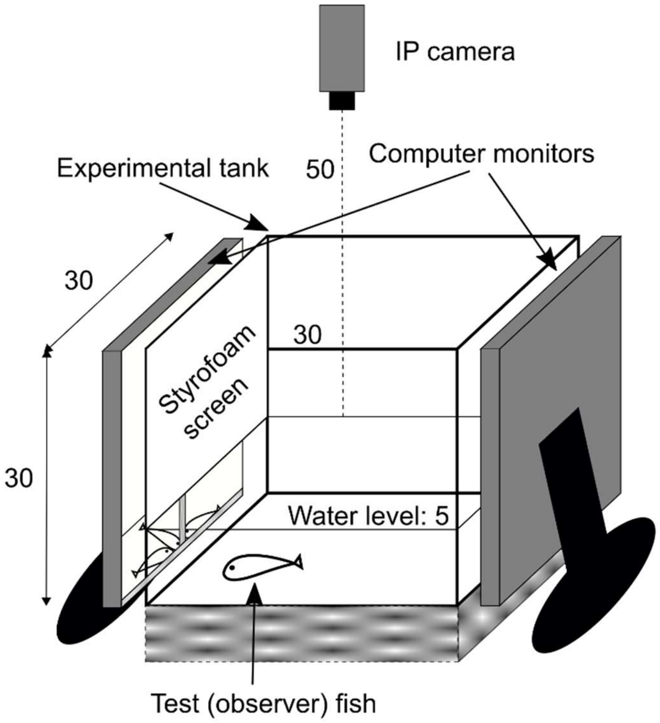
Figures

Figure 1:



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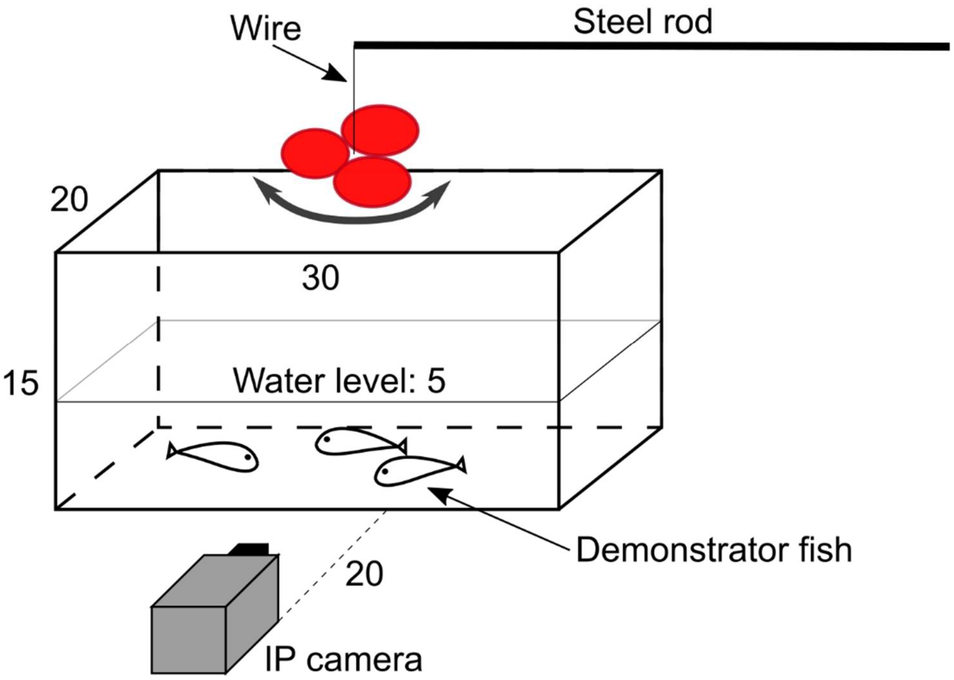

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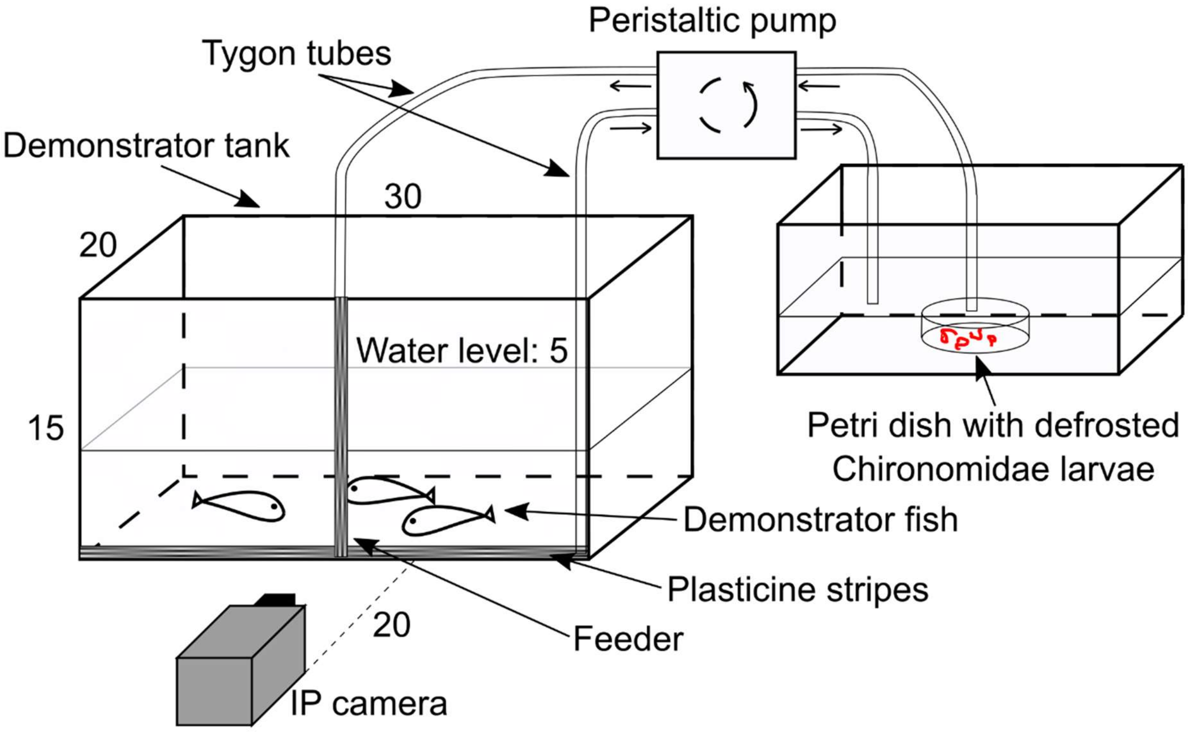

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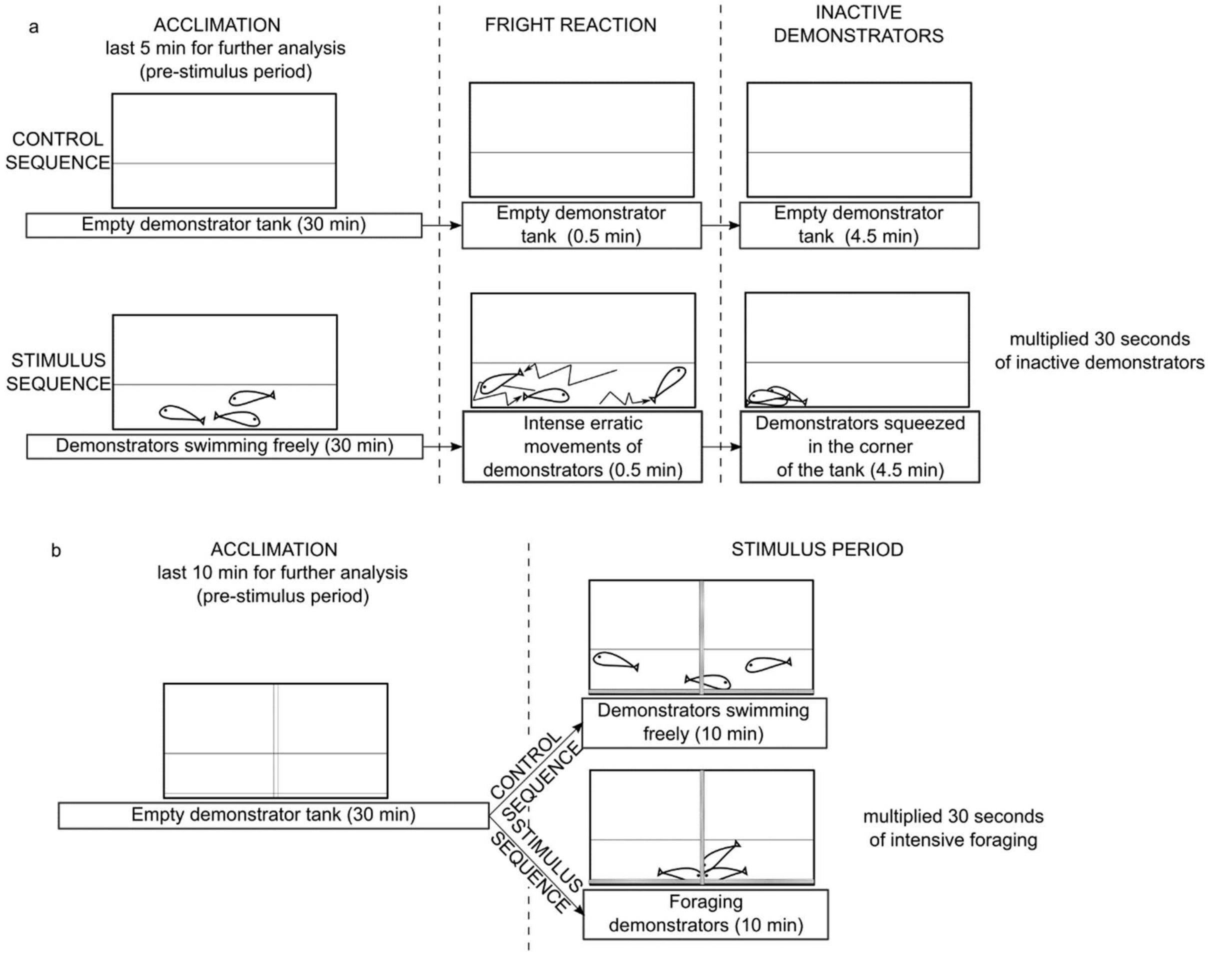

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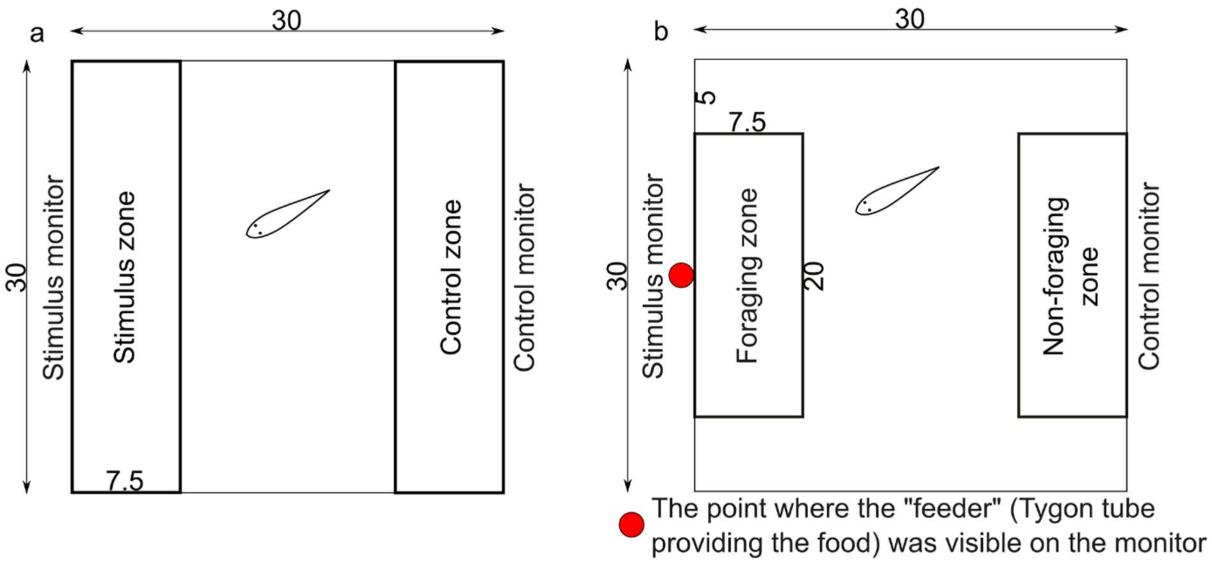

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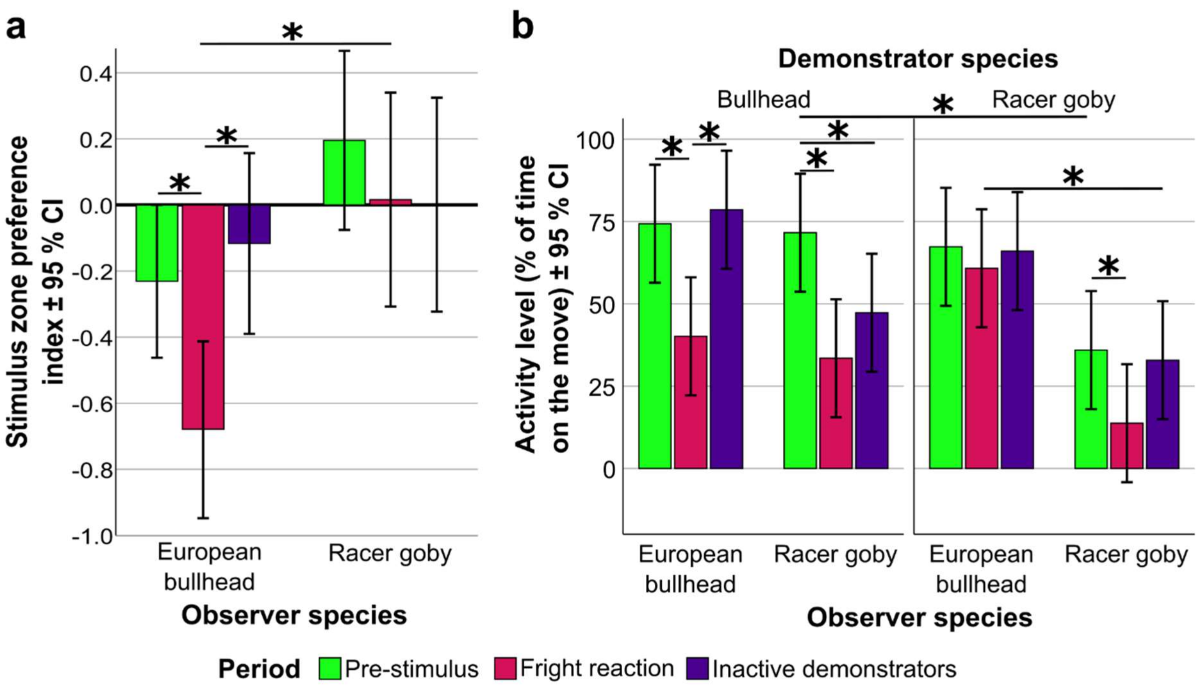

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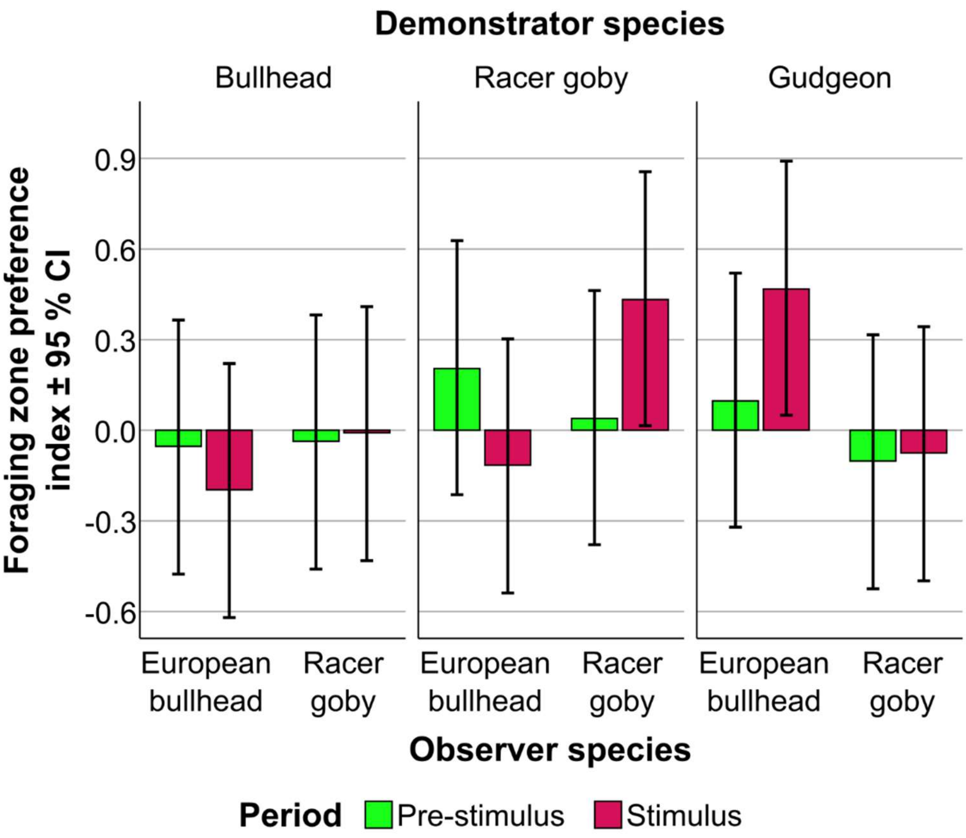

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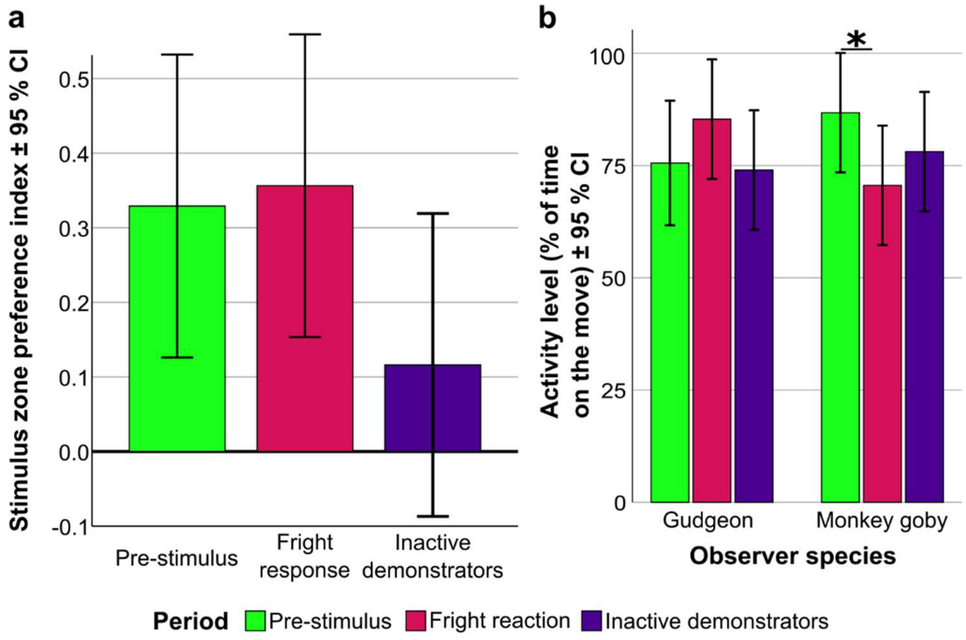

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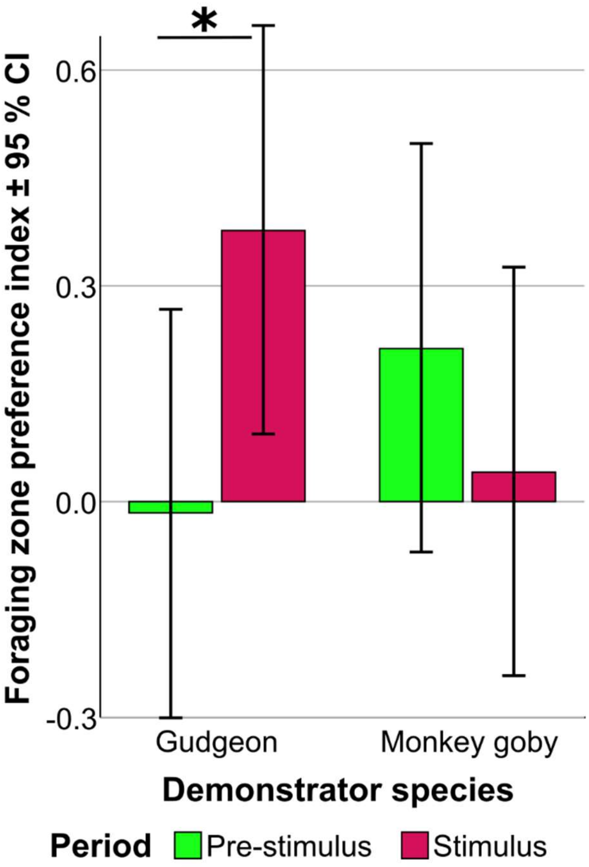
*
0.6
0.3
0.0
-0.3
Foraging zone preference index ± 95 % CI
Gudgeon
Monkey goby
Demonstrator species
Period
Pre-stimulus
Stimulus

Figure legends

Figure 1: Experimental setup to test public information use by fish. Two identical computer monitors were placed on the opposite sides of the experimental tank. The camera placed above the tank recorded fish behavior. An individual test fish (observer) was placed in the experimental tank for 30 min for acclimation. Simultaneously, we started to display the prepared videos (see “Stimulus preparation” section) on the monitors. Dimensions are given in cm

Figure 2: Setup to record fear reaction cues for Experiment 1. Three individuals of the same species (demonstrators) were placed in the demonstrator tank. Red plastic objects suspended by a wire from a steel rod were used to trigger a fright reaction of the demonstrators. See the text for further details. Dimensions are given in cm

Figure 3: Setup to record foraging cues for Experiment 2. Three individuals of the same species (demonstrators) were placed in the demonstrator tank. Food was provided to a Petri dish in a separate tank and sucked into the demonstrator tank with a Tygon® tube. The other tube pumped water out of the demonstrator tank to maintain the water level at 5 cm. Plasticine stripes made the food invisible in the videos. See the text for further details. Dimensions are given in cm

Figure 4: Video sequences used in Experiment 1 (a) and Experiment 2 (b). The video fragments were put together using smooth and slow transitions to minimize fish disturbances. The whole sequence was presented during each trial automatically. Procedures used to record each fragment are described in the “Processing the video recordings” section (Materials and Methods)

Figure 5: Zones set in the Ethovision XT ® 10.1 for video analyses. (a) Experiment 1. The “stimulus zone” faced the monitor showing demonstrators. (b) Experiment 2. The “foraging zone” faced the monitor with the stimulus video sequence. Dimensions are given in cm

Figure 6: The behavior of European bullhead and racer goby facing public information about danger in Experiment 1 (fear cues). (a) The “stimulus zone” preference index model estimates (means ± 95% CI) for the significant effect of observer species x period interaction (Table 1). (b) The activity model estimates (means ± 95% CI) for a significant observer species x demonstrator species x period interaction (Table 2). Asterisks indicate differences between particular groups after sequential Bonferroni corrected critical p-value

Figure 7: The behavior of European bullhead and racer goby facing public information from foraging demonstrators in Experiment 2 (foraging cues). The “foraging zone” preference index model estimates (means ± 95% CI) for the significant observer species x demonstrator species x period interaction (Table 3). There were no significant differences between groups after sequential Bonferroni correction for multiple analyses

Figure 8: The behavior of gudgeon and monkey goby facing public information about danger in Experiment 1 (fear cues). (a) The “stimulus zone” preference index results model estimates (means ± 95% CI) for the significant main effect of period (Table 4). There were no significant differences between groups after sequential Bonferroni correction for multiple analyses. (b) The activity model estimates (means ± 95% CI) for the significant observer species x period interaction (Table 5). Asterisk indicates differences between particular groups after sequential Bonferroni corrected critical p-value

Figure 9: The behavior of gudgeon and monkey goby facing public information from foraging demonstrators in Experiment 2 (foraging cues). The “foraging zone” preference index model estimates (means ± 95% CI) for a significant demonstrator species x period interaction (Table 7). Asterisk indicates differences between particular groups after sequential Bonferroni corrected critical p-value

Tables and table legends

Table 1: Linear Mixed Model on the "stimulus zone" preference index exhibited by the European bullhead/racer goby in Experiment 1 (fear cues).

Source	**df1**	**df2**	**F**	**P**
Observer Species (O)	1	37.004	8.132	0.007*
Demonstrator Species (D)	1	37.004	2.129	0.153
Period (P)	2	76.001	4.545	0.014*
O x P	2	76.001	3.243	0.045*

The observer species (European bullhead/racer goby) and demonstrator species (European bullhead/racer goby) were specified as between-subject fixed effects, period (pre-stimulus, fright reaction and inactive demonstrators) as a within-subject fixed effect and the fish ID as a random effect. Asterisks indicate significant effects at $p < 0.05$.

Table 2: Linear Mixed Model on the activity by the European bullhead/racer goby in Experiment 1 (fear cues).

Source	**df1**	**df2**	**F**	**P**
Observer Species (O)	1	36	10.286	0.003*
Demonstrator Species (D)	1	36	2.097	0.156
Period (P)	2	72	22.843	<0.001*
O x D	1	36	2.229	0.144
O x P	2	72	1.945	0.150
D x P	2	72	4.011	0.022*
O x D x P	2	72	3.208	0.046*

The observer species (European bullhead/racer goby) and demonstrator species (European bullhead/racer goby) were specified as between-subject fixed effects, period (pre-stimulus, fright reaction and inactive demonstrators) as a within-subject fixed effect and the fish ID as a random effect. Asterisks indicate significant effects at $p < 0.05$.

Table 3: Linear Mixed Model on the "foraging zone" preference index exhibited by the European bullhead/racer goby in Experiment 2 (foraging cues).

Source	**df1**	**df2**	**F**	**P**
Observer Species (O)	1	54	0.032	0.859
Demonstrator Species (D)	2	54	0.830	0.442
Period (P)	1	54	0.494	0.485
O x D	2	54	1.482	0.236
O x P	1	54	1.152	0.288
D x P	2	54	0.793	0.458
O x D x P	2	54	3.295	0.045*

The observer species (European bullhead/racer goby) and demonstrator species (European bullhead/racer goby/gudgeon) were specified as between-subject fixed effects, period (pre-stimulus and stimulus) as a within-subject fixed effect and the fish ID as a random effect. Asterisks indicate significant effects at $p < 0.05$.

Table 4: Linear Mixed Model on the “stimulus zone” preference index by the gudgeon/monkey goby in Experiment 1 (fear cues).

Source	**df1**	**df2**	**F**	**P**
Observer Species (O)	1	37.000	0.474	0.495
Demonstrator Species (D)	1	37.000	0.935	0.340
Period (P)	2	78.000	3.225	0.045*

The observer species (gudgeon/monkey goby) and demonstrator species (gudgeon/monkey goby) were specified as between-subject fixed effects, period (pre-stimulus, fright reaction and inactive demonstrators) as a within-subject fixed effect and the fish ID as a random effect. Asterisks indicate significant effects at $p < 0.05$.

Table 5: Linear Mixed Model on the activity by the gudgeon/monkey goby in Experiment 1 (fear cues).

Source	**df1**	**df2**	**F**	**P**
Observer Species (O)	1	36	0.001	0.982
Demonstrator Species (D)	1	36	0.230	0.634
Period (P)	2	74	1.333	0.270
O x D	1	36	0.388	0.537
O x P	2	74	8.976	<0.001*
D x P	2	74	1.315	0.275

The observer species (gudgeon/monkey goby) and demonstrator species (gudgeon/monkey goby) were specified as between-subject fixed effects, period (pre-stimulus, fright reaction and inactive demonstrators) as a within-subject fixed effect and the fish ID as a random effect. Asterisks indicate significant effects at $p < 0.05$.

Table 6: Linear Mixed Model on the "foraging zone" preference index exhibited by the gudgeon/monkey goby in Experiment 2 (foraging cues).

Source	**df1**	**df2**	**F**	**P**
Observer Species (O)	1	36.002	0.036	0.851
Demonstrator Species (D)	1	36.002	0.119	0.732
Period (P)	1	38.000	1.562	0.219
O x D	1	36.002	7.137	0.011*
D x P	1	38.000	10.218	0.003*

The observer species (gudgeon/monkey goby) and demonstrator species (gudgeon/monkey goby) were specified as between-subject fixed effects, period (pre-stimulus and stimulus) as a within-subject fixed effect and the fish ID as a random effect. Asterisks indicate significant effects at $p < 0.05$. Post-hoc tests showed no difference for an observer species x demonstrator species interaction.