Behavioural and trophic variation within a well-established invasive round goby population

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Abstract

An animal's behavioural traits can influence the outcomes of ecological interactions within their food-web, including what they eat, their vulnerability to predation and who they compete with. Despite this, few studies have directly measured links between among-individual behavioural and trophic variation. Invasive species like the round goby (*Neogobius melanostomus*) are often found to have consistent among-individual differences in behaviour within and between populations across their invasion front. Therefore, an individualized approach to invasive populations and their ecological interactions may be valuable to understanding their impacts on recipient ecosystems. Using non-lethal methods to measure trophic variation (i.e., stable isotope analysis via fin clips) and passive individual tagging, we analysed behavioural trait/personality variation and trophic variation to explore links between the two. Focusing on an established population of round gobies in the Guldborgsund strait in the southwest Baltic Sea, we found significant among-individual variation in bold-exploratory traits in novel environment and refuge emergence assays. We also found strong intraspecific trophic variation, with particularly high variation in carbon-12 – carbon-13 ($\delta^{13}C$) suggesting that individual round gobies differ in what they are feeding on and/or where they forage. Diet reconstruction results support previous studies showing that gastropods and bivalves are major contributors to their diet, but the large differences in isotope values suggest that individual variation influences how they interact with prey communities. There were few links between behavioural and trophic variation, nonetheless this study shows that measuring behavioural-trophic links is a viable approach for exploring if and how behavioural traits may influence individual-level ecological variation.

Keywords - boldness, diet specialisation, exploration, individualized niche, personality, isotopic niche

Introduction

Among-individual behavioural variation is regularly detected within a range of animal species, including highly impactful invasive species like the cane toad (*Rhinella marina*), mosquitofish (*Gambusia* spp.) and round goby (*Neogobius melanostomus*; Gosling 2008; González-Bernal et al. 2014; Behrens et al. 2020; Michelangeli et al. 2020). The component of intraspecific behavioural variation that is associated with among-individual differences is often termed 'animal personality', and may be estimated via repeated-measures experimental designs in one or more behavioural assays (Dall and Griffith 2014; Sánchez-Tójar et al. 2022). Boldness and exploration are broad classes of personality variation relating to risk-taking behaviour (referred to collectively here as 'bold-exploratory' traits), which can include responses to direct or indirect predation risk, and the willingness to enter or explore novel environments or to interact with novel objects or food items (Réale et al. 2007; White et al. 2013; Moran et al. 2016). Personality and bold-exploratory traits in particular have been linked to range of ecological processes, and their role in biological invasions and predator-prey interactions are increasingly of interest to ecologists (Wolf and Weissing 2012; Juette et al. 2014; Laskowski et al. 2022).

Personality variation can be important to the success or failure of invasions at multiple stages, including transport, introduction and establishment (Blackburn et al. 2011; Chapple et al. 2012). Post-establishment spread can also be facilitated by personality-biased dispersal, i.e., where individuals with certain behavioural traits (e.g., bolder, less social, or more active animals) can drive range expansion at invasion fronts (Cote et al. 2010; Thorlacius et al. 2015; Rehage et al. 2016). This trait-biased process can contribute to phenotypic-biases between populations across an invasion gradient in an 'invasion succession' (Gruber et al. 2017; Thorlacius and Brodin 2018). Populations across an invasion gradient may also experience varying environmental pressures that can influence the expression of behavioural traits in those populations, e.g., lower predation pressure and parasite/pathogen loads nearer invasion fronts (Torchin et al. 2003; Sih et al. 2010; Gendron et al. 2012), or higher intraspecific competition in denser established populations (Azour et al. 2015; Thorlacius et al. 2015). Therefore, behavioural variation may develop both within and between populations at different points in their invasive range, and the composition of individual behavioural phenotypes within each population may influence how they interact with local communities (Juette et al. 2014).

An individual’s behavioural traits can influence the strength and outcomes of their ecological interactions, and personality differences in boldness, exploration, activity, sociability and aggression have been linked to individual differences in foraging behaviour (Toscano et al. 2016). Bold-exploratory traits have been linked to differences in feeding rates, foraging strategy, and foraging habitat (Kurvers et al. 2009; Jolles et al. 2013; Patrick and Weimerskirch 2014; Jolles et al. 2016; although cf. Szopa-Comley et al. 2020). Furthermore, the composition of behavioural traits in a population may also influence how that population interacts with its food web, e.g., by influencing the strength and distribution of trophic interactions within their food web (Bolnick et al. 2011; Juette et al. 2014; Moran, Wong, et al. 2017). The nature and magnitude of an invasive predator’s impacts on an invaded community may therefore be influenced by personality variation. Invasive impacts in marine environments can be context dependent, where characteristics of the species, population and invaded environment are all likely to determine the potential impacts (e.g., effects on prey abundances, community structure and composition, on ecosystem function, etc.; see Thomsen et al. 2011). Behavioural trait composition may also be a factor that influences invasive predator impacts, and Juette et al. (2014) proposed that biases in bold-exploratory traits may influence foraging rates, diet breadth, and foraging areas, which may have potential impacts on prey abundances or the structure and composition of prey communities.

This study aims to quantify the links between personality variation and trophic interactions in invasive species, using the round goby as a case study. Native to the Ponto-Caspian region, round goby were first observed in the Baltic in 1990 (Skóra and Stolarski 1993). They have since been introduced again several times and also actively spread via secondary dispersal along the coastline (Sapota and Skóra 2005; Kornis et al. 2012; Kotta et al. 2016). Today they occur in most coastal areas of the Baltic Sea, often undertaking seasonal off-shore migration during the coldest months (Behrens et al. 2022; ICES et al. 2022). The round goby can reach extremely high abundances in the Baltic, for example densities of 1.9 fish/m^2 were recorded in Guldborgsund, Denmark, approximately 4 years after their first detection (Azour et al. 2015). As a result, they can have major impacts on the abundance and composition of local benthic communities through resource competition and direct predation (Kipp et al. 2012; Pennuto et al. 2018; van Deurs et al. 2021). More established populations also tend to be characterised by high intraspecific resource competition, which appears to be associated with a broader dietary niche than new populations (Herlevi et al. 2018; Nogueira Tavares et al. 2022). Their bold-exploratory traits have also been relatively well studied (Marentette et al.

2011; Marentette et al. 2012; Flink et al. 2017; Loftus and Borcherding 2017; Behrens et al. 2020). There is also evidence of phenotypic variation between Baltic populations in predator responses (Galli et al. 2023), and studies showing behavioural variation across their invasion fronts that appears to be linked to personality-biased dispersal (Myles-Gonzalez et al. 2015; Thorlacius et al. 2015; Thorlacius and Brodin 2018). These studies suggest that populations nearer the invasion front may be bolder, more active and less social (although cf. Groen et al. 2012), suggesting the personality composition of populations may be a factor that could influence their impacts across their invasive range.

Stable isotope analysis (‘SIA’) is a useful tool to analyse trophic variation concurrently with behavioural traits. SIA may be useful for characterising a species’ diet and describing trophic relationships at population and individual levels (Post 2002; Boecklen et al. 2011; Hückstädt et al. 2012). As organic tissue is consumed and assimilated into the tissue of higher level consumers/predators, the ratios of carbon-12 – carbon-13 (hereafter ‘δ^{13}C’) and nitrogen-14 – nitrogen-15 (hereafter ‘δ^{15}N’) are enriched by a relatively consistent ‘fractionation’ or ‘discrimination’ factor (Caut et al. 2009; Britton and Busst 2018). Isotopic variation among species, populations or individuals can then be used to describe trophic relationships between consumers and their prey, and also to infer links between consumers and primary carbon sources (e.g., phytoplankton, macro-algae, etc.; Layman et al. 2012). Assimilation rates may vary, but isotope ratios of softer tissues like skin/scales, muscle and fins may be used to infer diet variation over periods of several weeks to months (Thomas and Crowther 2015; Britton and Busst 2018). Fin tissues are particularly useful for non-lethally measuring isotopic variation in fish, providing that within-individual/-fin isotopic variation is accounted for (Jardine et al. 2011; Hayden et al. 2015). Samples can be taken with relatively little stress to the fish, and extremely small amounts of tissue are required (e.g., 0.5 mg or less of dry tissue per replicate). The round goby has also been the subject of previous SIA analyses, including evidence of isotopic variation within and between populations (Brush et al. 2012; Brandner et al. 2013; Pettitt-Wade et al. 2015; Herlevi et al. 2018; Feiner et al. 2019; Verstijnen et al. 2019; Nogueira Tavares et al. 2022). Their diet has also been characterised in regionally-relevant studies (e.g., Oesterwind et al. 2017; van Deurs et al. 2021), and species-specific discrimination factors have been estimated (Poslednik et al. 2023). Therefore, SIA is likely to provide a robust tool for describing the species’ diet in the Baltic region.

Very few studies have directly measured how laboratory-measured behavioural traits may correspond to isotopic/trophic variation in wild animal populations, with mixed results. For example, early studies failed to identify links between laboratory-measured dominance behaviours and trophic position in an invasive crayfish (*Faxonius rusticus*; Glon et al. 2016; Adey and Larson 2020). Another study of three-spined sticklebacks (*Gasterosteus aculeatus*) successfully found links between boldness, sex and δ^{13}C values (Theódórsson and Ólafsdóttir 2022), suggesting that links between among-individual variation linked to ecological segregation may be detected using this approach.

Therefore, the overarching objective of this study is to concurrently measure both individual behavioural and trophic state variation, using round gobies collected from a well-established Baltic Sea population. Our specific goals of this study were to:

1. *Measure among-individual variation in bold-exploratory traits and trophic state.* We expected to find repeatable differences in bold-exploratory behaviours, as these are common in animals including the round goby (Gosling 2008; Bell et al. 2009; White et al. 2013; Myles-Gonzalez et al. 2015; Behrens et al. 2020). Similarly, we expected to find among-individual trophic variation within the population (i.e., in δ^{13}C and δ^{15}N), as substantial within-population trophic variation has been previously found in established round goby populations (e.g., Herlevi et al. 2018; Feiner et al. 2019). In addition, we explored effects of state variation (body size, body condition and sex) as potential sources of behavioural and trophic variation.
2. *Explore whether individual behavioural variation may be linked to their trophic interactions.* We did this by investigating potential drivers of trophic variation in the population using SIA analysis of prey taxa and isotope mixing models to estimate the relative contributions of prey groups to round goby diets. Next, we calculated correlations between individual isotopic values and behavioural traits, to identify if any specific traits are linked to their trophic interactions.

Methods

I. *Field sampling*

Round gobies and potential prey items were sampled in June 2020 from the shallow brackish estuary Guldborgsund in the western Baltic (54.69645°, 11.84067°; Fig. 1). Guldborgsund was one of the first Danish areas to be invaded (est. ca. 2009). The population in this area is well-established and self-sustaining , and characterised by a high population density where gobies experience substantial intraspecific resource competition and exert strong predation pressure on benthic fauna (Azour et al. 2015; van Deurs et al. 2021). Sampling took place over a 2 ha. area of coastal habitat (100 x 200 m, depth < 2.0 m, sandy and scattered-boulder substrate). This habitat is well-suited to this species due to the abundance of their preferred prey items (e.g., gastropods and bivalves; van Deurs et al. 2021), and because gobies have a strong affinity to rocky structures for shelter, food and nesting sites (Lynch and Mensinger 2012; Christoffersen et al. 2019). Round gobies are most active in coastal/inlet areas over spring-summer periods, particularly from April to June in the Baltic region as fish have migrated into shallow waters to prepare for/initiate spawning (Brauer et al. 2020; Behrens et al. 2022). As isotopic variation in soft tissues generally reflects diet over several weeks up to months (Thomas and Crowther 2015), we assume here that variation in round goby tissue collected in June is primarily linked to their local diet within the estuary.

Round gobies were collected using a combination of passive nets and traps deployed overnight (for details see supplementary materials S1). Multiple methods were used to minimise personality-biased sampling (Biro and Dingemanse 2009; Michelangeli et al. 2016). Fish below 80 mm total length (TL) were excluded to focus on variation in their adult diet, as this species' undergoes an ontogenic transition towards feeding on more hard-bodied prey items at approximately this size (Andraso et al. 2011). The mean size of fish at tagging was 13.19 cm [range; 8.0 – 17.3]. Fish (n = 55) were transported to DTU Aqua fish stable facility (Fig. 1, Lyngby, Denmark). Upon arrival, the fish were randomly allocated among three enriched circular 800 L holding tanks receiving filtered, recirculated, well-aerated seawater at 10 °C, and a salinity of 16 ± 1 ppt, and maintained under a 12:12 hr light:dark cycle, with inclusion of a half-hour dawn and dusk. To enhance welfare and provide hiding places, artificial seagrass was on the tank bottoms. Ammonium (NH_4+), nitrite (NO_2-), nitrate (NO_3-) and pH of the water were measured twice a week (Testlab Marin; JBL, Neuhofen, Germany). They were fed to satiation three times per week with commercial high-nutrition pellet fish feed (3mm Ivory Ex composite pellets, Aller Aqua, Denmark). The salinity used is within the natural range of the source location (Feistel et al. 2010), and is well within a salinity range where the species is able to thrive (Behrens, van Deurs, et al. 2017; Puntila-Dodd et al. 2021).

To capture a representative cross-section of the benthic community, including infauna, sessile taxa and mobile taxa, prey were collected using a combination of methods; benthic cores; push net samples; 50 x 50 cm quadrat samples; and bycatch from baited box/cylinder traps (also see supplementary materials S1). Samples were rinsed through a 0.5 mm sieve with deionised water. Eight replicates of each sample type were collected and placed on ice for transport (approx. 2 hrs) and subsequently frozen at -40 °C before further processing. Primary carbon sources were also sampled to provide additional context to any observed isotopic variation. Replicates of any dominant type of algae were collected by hand. Coarse particulate organic matter ('CPOM') was collected from core and quadrat samples, which was primarily woody/leafy detritus from terrestrial and riparian vegetation. To represent the phytoplankton community, three replicate samples were taken of fine particulate organic matter ('FPOM') using water collected from the deepest area of the sampling area (approx. 2 m). Water was pre-filtered through a 47 µm sieve and vacuum filtered onto Whatman GF/F glass microfiber filters (GE Healthcare, Denmark A/S). This represents a 0.7 – 47 µm FPOM fraction that captures a large component of the local phytoplankton community in late spring-early summer. Samples and filters were then frozen at -40°C before further processing.

II. Individual tagging, tissue sampling and physical measurements

On day two after returning to the laboratory, individual fish were tagged, and tissue samples were taken from all individuals. Small passive integrated transponder ('PIT') tags (12 × 2 mm, 0.1 g, Oregon RFID Inc.) were injected into abdominal cavities with a syringe implanter (MK25, Biomark Inc.) under anaesthetic following standard procedures (using MS-222, Acros Organics, UK; Jørgensen et al. 2017). These small passive tags do not affect survival and growth in round goby (Ruetz et al. 2006; Cookingham and Ruetz 2008), unlike larger (e.g. acoustic) tags that may have an impact on the fish (Behrens, Svendsen, et al. 2017). Caudal fin tissue samples were taken as a low-impact non-lethal alternative for SIA, with values that closely compare to muscle tissue (Jardine et al. 2011). The extreme outer edge (<5 mm) of fins was taken to standardise sampling between individuals, and each fin was then split into three replicates that were stored and processed separately to account for within-fin variation (Hayden et al. 2015; Britton and Busst 2018). Fish had their TL measured before tissue sampling and tagging, and were inspected for eye fluke parasites that may influence their behavioural responses (Flink et al. 2017). The full process took < 60 s per fish. Sexing and weighing were completed several days later to avoid further stressing fish during this procedure.

Of the 55 fish returned to the lab, 34 survived until the end of the full 30-day experimental period. Given this level of attrition, we have assessed potential impacts of survivorship bias on our findings and conducted an additional pilot experiment to determine whether tagging/tissue sampling may have influenced survival. No effects of these procedures on survival or health were detected, and previous and subsequent experiments have had substantially lower mortality using similar or identical procedures (for further analysis and discussion see supplementary materials S2).

III. Behavioural analysis

Two forms of behavioural trials were conducted, referred to here as the *Activity* and *Exploration* assay. Trials started 11 days after tissue sampling/tagging to allow a recovery period, and trials were repeated three times at one-week intervals. Before and between behavioural trials, fish remained in their holding tanks (as described above).

The *Activity* assay used an open field design to measure bold-exploratory traits and activity in a novel environment (Fig. 2A; Toms et al. 2010). Individuals were selected blindly from housing tanks using large dip nets and placed into arenas using smaller plastic transport tubs (24 x 34 cm) to minimise stress and ensure that fish remain entirely in water throughout the experimental process. Eight identical opaque white PET plastic boxes were used as arenas (32.25 x 49.25 cm, water height of 21 cm). Arenas were arranged in a 2 x 4 grid under a laboratory fluorescent light in an area separated by a curtain, so light conditions were consistent (~ 45 lux) and external sound/light effects were minimised. Behaviour was recorded for 20 minutes following a 5-minute acclimation period using a modified web camera positioned centrally 2 m above the arenas (Logitech BRIO 4K Ultra HD webcam, Logitech, Switzerland). To avoid carryover effects, arenas are emptied, rinsed with deionised water and filled with new filtered water between each trial.

Movement was tracked using Toxtrac v2.90 (Rodriguez et al. 2018). Arenas were manually calibrated using the pixel/mm ratios for each arena, calculated via ImageJ v1.52a (Schneider et al. 2012). Variables relating to activity and edge/centre use were extracted (see Table 1), where more activity in a novel environment and greater use of more exposed central areas are generally considered to be bolder/more exploratory behaviour (Moran, Mossop, et al. 2017; Moran et al. 2021).

The *Exploration* assay used a refuge emergence design to measure an individual's willingness to enter and explore a novel environment (Fig. 2B; Toms et al. 2010). Individual fish were placed into a 32 x 16 cm enclosed refuge at one end of a narrow arena (135.5 x 16.0 cm, water depth = 13.5 cm). Five parallel exploration arenas were used, with black opaque acrylic walls between each arena, and sand lining the bottom of the arenas (0.5 – 1 mm white grain sand, 2 – 3 mm depth). After an extended 10-minute acclimation period, the partitions separating the refuge from the arena were removed simultaneously by an observer positioned behind the refuges, so their movement was not visible to the fish. Behaviour was filmed as above for 45 minutes, with the camera positioned centrally 1.5 m above the arenas. Arenas were connected to an inflow from the recirculation system to maintain constant water quality throughout the experiment (approximately 5.5 mL/s inflow from the refuge end). Arenas were completed flushed with filtered water between trials to avoid carryover effects.

Behaviour was manually scored from videos, with response variables focusing on their tendency to emerge and explore to the endpoint of the arena (Table 1). Being faster to emerge and/or explore a novel environment is generally considered to be bolder/more exploratory behaviour (Moran, Mossop, et al. 2017; Moran et al. 2021). Fish were fed on the day before each trial to standardise satiation levels. After each assay, fish had their PIT tag recorded to confirm their individual identity and were checked for any physical injuries or lethargy. Both assays were repeated three times at one week intervals, with *Exploration* assays run two days after *Activity* assays to limit carryover effects between trials (Bell 2013).

IV. Stable isotope processing

Unless otherwise stated, isotope samples were prepared following standard SIA methods for marine aquatic food webs (Jardine et al. 2003). Fins were thoroughly rinsed with purified DI water to remove any surface contamination. Prey items were picked out of bulk samples under magnification, rinsed and sorted into taxonomic groupings to at least family or order level where possible (see groupings in supplementary materials S3). Soft tissue from gastropods was removed from shells, as carbon-based precipitates reflect the isotopic ratios of the inorganic environment (Post 2002). Guts were dissected out where possible and soft tissue was used in analysis for the majority of taxa, as depuration of gut contents was not possible (Curtis et al. 2017). Smaller prey items such as ostracods, chironomids were used whole and multiple individuals were pooled as required to ensure sufficient biomass for analysis. Three independent replicates per taxa were produced. Three replicates of major primary producers were also rinsed and any invertebrates were removed.

Fin, prey and primary producer samples (including FPOM packed filters) were each dried at 60 °C in an oven for 48 hours. Fins were not homogenized before packing, as there was limited biomass available and replicate samples per individual were able to account for and estimate residual/within-tissue variation. All other samples (excluding filters) were homogenized by grinding into a fine powder. Fin and prey samples were weighed (0.5 mg ± 0.1 for all samples) and double encapsulated in 4 x 6 mm tin capsules (Elemental Microanalysis Ltd, UK) to ensure complete combustion. Algae and plant samples were encapsulated in single 4 x 6 mm capsules, while sections of FPOM filters were double encapsulated (5 x 8 mm).

Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ isotope ratios and N% and C% by mass, using a FLASH HT Elemental Analyser interfaced via a ConFlo IV Universal Continuous Flow Interface to a DELTA XP Isotope Radio Mass Spectrometer (Thermo Fischer Scientific, USA), at the University of East Anglia Stable Isotope Laboratory (Norwich, UK). Masses (mg) were determined via the Flash HT signal count relative to a sulphanilamide reference standard, and N% and C% calculated as a percentage of the total initial sample mass. Data for $\delta^{13}C$ and $\delta^{15}N$ are calculated by $\delta^{13}C/\delta^{15}N = (((R_{measured}/R_{reference})-1) \times 1000)$ and are expressed in per mille (‰) relative to the isotopic ratio of Vienna Pee Dee Belemnite standard ($R_{VPDB} = 0.0111797$) for carbon and atmospheric N_2 ($R_{Air} = 0.0036765$) for nitrogen.

V. *Statistical analysis*

All analyses were conducted in R (v4-2.3, R Core Team, 2013). All data, code, and models from these analyses are also available via the Open Science Framework (https://osf.io/rnz7q).

The distributions of behavioural variables were assessed and transformed where required (see Table 1). Emergence and endpoint latencies were heavily bimodal, and therefore converted to binary response variables based on the median score from the cohort (as per Moran, Mossop, et al. 2017). Repeatability was estimated for each variable (i.e., the proportion of total variance associated with among-individual variance, via the ‘rptR’ package; Stoffel et al., 2017). Adjusted repeatabilities were also calculated to remove the potential influence of experimental grouping factors on either the among-individual or residual variance components (Nakagawa and Schielzeth 2010). Grouping factors were experimental arena (ArenaID), holding tank (TankID), replicate number (TrialDay), and trial set (TrialRound). A subset of fish was excluded from analysis as they were found with signs of physical injury or lethargy in post-trial inspections (i.e., data for fish G08, G23, G32 and G47 were entirely excluded, and data for G21, G40 and G41 were partially excluded from week 2 and 3 trials). This was necessary to limit the effects of injuries or illnesses contracted after they arrived in the laboratory on the observed behavioural variation.

The influence of state variables (i.e., TL, sex, and condition) on each repeatable behavioural variable were analysed using LMM/GLMM models (via package “lme4”, v1.1-27.1, Bates et al. 2015). A population-specific condition factor (‘CondManual’) was calculated per Galli et al., (2023), to ensure the condition factor was independent of body length. Unlike for repeatability estimates above, TrialDay was included as a fixed effect here to allow us to estimate acclimatisation effects. Continuous fixed effects (i.e., TL, CondManual and TrialDay) were Z-scaled to help cross-interpretability of effect estimates (Schielzeth 2010). Models initially included ArenaID, TankID and TrialRound as potential random effects, but were excluded from final models where they explained extremely little or no variance. Relationships between behavioural variables were then explored via Spearman (non-parametric) correlations using untransformed variables.

Stable isotope values may be influenced by the proportion of lipid in tissues due to differential fractionation of carbon isotopes during lipid synthesis, so a lipid normalisation factor may be applied where samples’ C:N mass ratios include values over 3.5 (as is the case here; Skinner et al. 2016). Round goby $\delta^{13}C$ values were normalized via equations from McConnaughey and McRoy (1979), which are parametrised based on values for fish tissue specific to the Baltic Sea region (Kiljunen et al. 2006). This correction was not applied for prey items as our focus is on characterising round goby diets, so it is assumed here that both prey lipids and proteins are assimilated into goby tissue (as in Newsome et al. 2014).

Stable isotope Bayesian mixing models were used to characterise the round goby diets (via package “MixSIAR”, v3.1.12; Stock et al. 2018). Mixing models used uninformative priors and 3 chains (length = 300,000, burn-in = 200,000, thinning = 100), and convergence was confirmed using internal tools (e.g., via Gelman-Rubin convergence diagnostics). *FishID* was included as a random factor in models, to account for the individual-level replicates. A subset of taxa groupings were included as potential diet components, focusing on those groups that have previously been found in round goby stomach contents within this specific estuary (for full list see supplementary materials S3; as per van Deurs et al. 2021). These taxa were further pooled into five class-level groupings, to reduce the number of potential diet sources relative to isotopic tracers (Phillips et al. 2005; Phillips et al. 2014). Final groups were Bivalvia, Gastropoda, Malacostraca (decapods only), Malacostraca (other), Actinopterygii. As soft-bodied prey may be underrepresented in gut content analysis (Brush et al. 2012), an additional expanded model was run to explore the potential role of soft-bodied prey in the population's diet (see details in supplementary materials S4). Decapods were separated from other Malacostraca crustaceans as their isotope values suggest they feed at a higher trophic level than the comparatively smaller isopods or amphipods (see Fig. 3). One fish (G34) was excluded from diet analyses, as their $\delta^{15}N/\delta^{13}C$ values were extreme outliers (Z-scores >3) and their trophic position relative to the prey community and goby population suggests that they are likely a recent immigrant into the estuary (also see Fig. 3).

Discrimination factors represent the fractional change in isotope values from source to consumer tissues ($\Delta^{15}N$ and $\Delta^{13}C$; Caut et al. 2009; Phillips et al. 2014). The selection of discrimination factors can also influence the outcomes of diet reconstruction models (Bond and Diamond 2011). Therefore, round goby-specific $\Delta^{15}N$ and $\Delta^{13}C$ values were used here for mixing models, which were recently estimated by Poslednik et al. (2023; i.e., $\Delta^{15}N$: 4.04 ‰ ± 2.56 SD; $\Delta^{13}C$: -0.4 ‰ ± 2.56 SD). Sensitivity analyses were also conducted to assess the influence of this choice on our results, instead using general values from Post (2002) that are commonly applied in SIA analyses (see supplementary materials S4).

Variance components for $\delta^{15}N$ and normalized $\delta^{13}C$ values of round gobies were assessed using LMM models, to estimate the variance associated with FishID (V_{FishID}) and residual variance (V_r). Initial models included only FishID as a grouping factor. Repeatabilities of isotope values by FishID were also calculated as an estimate of the proportion of variance associated with among-individual variation. Expanded models were then used to test for state effects on isotopic variation, using TL, sex, and condition as fixed effects with continuous variables Z-scaled.

Finally, links between behavioural and trophic variables were investigated via Spearman correlations. Behavioural variables analysed were: (i) the individual's average behavioural score for all repeatable variables across the three trials (i.e., to detect correlations between among-individual behavioural variation and trophic state); and (ii) the behavioural scores from the first replicate only (i.e., to detect correlations between behavioural phenotypes and trophic state). First replicate scores were included as this allowed the maximum number of fish to be included, and also reflects the scoring of behavioural phenotypes that is most closely proximate to sampling (i.e., the score that is least influenced by acclimation to the laboratory or assay). As these analyses are exploratory, corrections for multiple correlations have not been used (or above), so any single significantly non-zero correlation should be interpreted cautiously.

VI. Ethical note

All laboratory experiments were authorized by the Danish Animal Experiments Inspectorate (Dyreforsøgstilsynet, under permit 2017-15-0201-01282 and its extensions). The welfare of animals was closely monitored at all times, and the sample size was minimized based on existing power analyses (e.g., Dingemanse and Dochtermann 2013). Fish were transported in large continuously aerated holding tanks and do not appear to show any negative effects of transport. Holding facilities were closely controlled to maintain optimal conditions, including 24-hour monitoring. For procedures that required fish to be held outside of water (i.e., tagging/fin clips), fish were anesthetized following well-established procedures that appear to show negligible impacts on their welfare. Notably, there was relatively high mortality over the full experiment (~40%) relative to comparable experiments with this species, which did not appear to be associated with experimental procedures but did highlight a need for refinement in future experiments (see detailed discussion in supplementary materials S2). All behavioural experiments were non-invasive, and all steps were taken to minimize any stress due to movement between tanks/arenas (e.g., the use of small transport tubs to avoid removing fish from water at any time). Following this study, all remaining animals were held in the facility and reused for further experimental and teaching purposes as required.

Results

I. Behavioural effects

Behavioural variables in both the *Activity* and *Exploration* assays were repeatable (Table 1), showing there were consistent behavioural differences among individuals. Overall raw and adjusted repeatabilities were closely comparable, although adjusted estimates appeared to be marginally higher in some *Activity* variables (e.g., *avespeed_mob*, $R_{Raw} = 0.24$ [0.02, 0.43], $R_{Adj} = 0.34$ [0.12, 0.52]), suggesting that some behavioural variance is associated with experimental factors and/or habituation effects.

State and habituation effects influenced several *Activity* variables. Condition had a significantly non-zero negative effect on time frozen, a non-significant marginally positive effect on distance moved, and no effect on average speed (Table 2). This suggests a weak effect where higher condition fish were more active, driven by those fish spending more of their time in motion. Centre use was associated with TL, where fish with a larger body size tended to use the central/exposed areas of the arena more than smaller fish (Table 2). Sex had an influence on distance, speed, and time frozen, where male fish were generally more active and faster moving (Table 2). Although notably, the sample size for female fish in this analysis is low (i.e., $n_{female} = 6$, $n_{male} = 37$), therefore any sex effects should be interpreted cautiously. Finally, replicate number (i.e., TrialDay) was associated with reductions in distance moved and speed, and increased time frozen (Table 2), suggesting a habituation effect where fish became less active in a novel environment over repeated trials. In contrast, there were no state or habituation effects in the *Exploration* assay.

There were strong correlations between most behavioural variables, including between the two behavioural assays (Table 3). Fish that moved a greater distance in *Activity* trials both spent less time frozen and tended to move around the arena faster when in motion. Those fish that were more active in the *Activity* assay were also faster to emerge and reach the endpoint of the *Exploration* assay (i.e., had lower emergence and endpoint latencies). Centre use was not significantly correlated with any other variable.

II. Trophic effects

An SIA biplot was produced to show variation in round goby $\delta^{13}C$ and $\delta^{15}N$ values relative to their community (Fig. 3). Round gobies had an mean $\delta^{13}C$ value of -15.46 ‰ (range: -17.95 – -13.12), and $\delta^{15}N$ value of 10.94 ‰ (range: 9.49 – 12.44). Among-individual differences accounted for the majority of variance in $\delta^{13}C$ (V_{FishID} = 1.65 [1.12, 2.45]; V_r = 0.13 [0.1, 0.18]; repeatability = 0.93 [0.88, 0.95]), and in $\delta^{15}N$ (V_{FishID} = 0.42 [0.28, 0.63], V_r = 0.08 [0.06, 0.1]; repeatability = 0.85 [0.76, 0.90]). This shows that there are significant among-individual differences in isotope values, relative to within-individual (i.e., within-tissue) variation. Isotopic values of individuals are the result of isotopic turnover and tissue assimilation over periods of weeks or months; therefore, this variation may be associated with consistent differences in diet between individuals in their relatively recent history.

The diet reconstruction model suggested that both gastropods (42.4% [23.8%, 61.5%]) and bivalves (30.4% [15.4%, 48.9%]) represented the largest proportion of diets (see Fig. 4). In contrast decapods (13.2% [2.2%, 29.5%]), amphipod/isopod crustaceans (9.8% [3.1%, 21.0%]), and fish made up smaller proportions (4.2% [0.6%, 11.1%]). However, sensitivity analysis suggests that these proportions will differ when using generic $\Delta^{15}N/\Delta^{13}C$ values in models (see supplementary materials S4). Furthermore, the inclusion of additional soft-bodied taxa in analysis suggests that insect larvae and polychaetes may have some role in their diet, if they are preyed upon by the Guldborgsund population (also see supplementary materials S4). Estimates of individual diet composition also varied, for example the proportion of bivalves in diets ranged from 1.6% [0%, 6.8%] (fish ID: G30) to 89.6% [72.3%, 99.4%] (fish ID: G54). These individual estimates are likely to be extremely sensitive to model inputs (e.g., selection of diet components, discrimination factors, etc.), so minimal weight should be placed on specific estimates. Nonetheless, the broad range in estimates for each fish does demonstrate that diet proportions estimated via SIA can be strongly impacted by intraspecific variation in the round goby.

There were no significant effects on $\delta^{15}N$ due to length ($Estimate_{TL(Z\text{-}scaled)}$ = - -0.09 [-0.31, 0.12]), or condition ($Estimate_{CondManual(Z\text{-}scaled)}$ = 0.03 [-0.16, 0.22]). There was a non-zero positive sex effect for males ($Estimate_{Sex(male)}$ = 0.79 [0.25, 1.33], P = 0.008) suggesting males may feed at a higher trophic level than females. For $\delta^{13}C$, there were not any non-zero effects due to length ($Estimate_{TL(Z\text{-}scaled)}$ = -0.12 [-0.31, 0.12]), sex ($Estimate_{Sex(male)}$ = -0.52 [0.25, 1.33]), or condition ($Estimate_{CondManual(Z\text{-}scaled)}$ = -0.02 [-0.16, 0.22]). There was a negative correlation between $\delta^{13}C$ and the average centre use score across trials (see Table 4), suggesting that fish that consistently used centre areas more in the *Activity* trial had lower $\delta^{13}C$ values than fish that preferred edge areas. Although this was not significant based on the trial 1 scores alone, and there were no significant correlations between $\delta^{13}C/\delta^{15}N$ and any other measure.

Discussion

The behavioural assays captured repeatable among-individual variation in bold-exploratory behaviours. Traits such as refuge emergence, activity in a novel environment, and centre/edge use all involve some level of risk-taking. Increased risk-taking in these contexts (i.e., greater use of central/exposed areas, more activity, faster emergence) generally infers a higher danger of predation/mortality, as a trade-off with greater access to food or reproductive resources (Luttbeg and Sih 2010; Moran et al. 2021). SIA analysis also found substantial variation in isotopic values between individual gobies. This is in line with previous studies that have found broad isotopic niches, particularly in established round goby populations (Herlevi et al. 2018; Nogueira Tavares et al. 2022), suggesting that there may be a substantial degree of resource segregation within the population. In addition, we did not find strong links between trophic and behavioural variation, and there is inconclusive evidence about the factors (e.g., ontogenic, sex, behavioural traits, etc.) that may underlie trophic variation in the Guldborgsund population.

Round gobies from Guldborgsund showed repeatability in six behavioural variables, most of which were correlated. Faster emergers and explorers in the *Exploration* assay were also more active, faster moving and spent less time frozen in the *Activity* trials. Behavioural measures are often correlated, such that individuals may be generally bolder across several contexts, although different measures may also vary independently (White et al. 2013). In contrast, centre area use was not correlated to other variables, suggesting that this behavioural trait may be under distinct selective and/or environmental pressures in this population.

State variables had some influence on behavioural variation. Female fish were less active, which is consistent with previous studies (e.g., Marentette et al. 2011). The low representation of female fish in our sampling and in many other studies could be driven by underrepresentation of females in passive gear (as observed in Žák et al. 2018), and/or by male biases that appear to exist in many invasive round goby populations (Kornis et al. 2012). This female bias was further exacerbated by poor survivorship of female fish in this study. Nonetheless, collecting sex-representative samples of wild populations should be an emphasis of round goby field studies. Condition had limited effects on behaviour, with a small negative impact on time frozen (i.e., fish in better physical condition spent more time moving around the arena than low condition individuals). This is in contrast to a recent meta-analysis, which suggests lower physical condition tends to be associated with greater levels of risk-taking (Moran et al. 2021). Total length was also associated with increased boldness in centre use but had no influence on other measures. This is consistent with some other studies from the Baltic showing limited links between size and behaviour in adult round gobies (Thorlacius et al. 2015; Thorlacius and Brodin 2018). Although size-dependent differences in activity have been detected in a similar study from closely related populations (see Galli et al. 2023), suggesting that patterns of state-dependent variation in behaviour may vary from population to population. Finally, there were strong effects of replicate in the *Activity* assay, where fish generally reduced their activity over the three trials. This does not appear to be related to changes in condition, as the cohort that survived through to the third activity trial appear to have slightly gained weight (i.e., mean weight was 35.71g at trial 1 versus 36.32g at trial 3). This instead suggests that the 'novelty' of the assay environment is reduced over the repeated trials, e.g., due to their previous experience of the environment and perceived lower danger of predation and/or potential for resource acquisition in subsequent trials, and fish respond by becoming less active and exploratory.

Diet reconstruction via MixSIAR appears to perform well at describing the population-level dietary composition. The choice of discrimination factors was influential, as is common in diet reconstruction analyses (Bond and Diamond 2011). Species-specific discrimination factors via Poslednik et al., (2023) appear to have performed well, and potentially better than generic values (for details see supplementary materials S4). Furthermore, the diet reconstruction result in the main text are in line with previous studies in the region (e.g., van Deurs et al. 2021), and highlight the importance of hard-bodied invertebrates to the adult diet of round gobies. Although importantly, our analysis is based on a subset of prey taxa based on previous gut-content analysis, which tends to underrepresent soft-bodied prey (Brush et al. 2012). Therefore these results may not be capturing a significant proportion of diet linked to other common taxa in the estuary (e.g., polychaetes, chironomids etc.), which are known to be part of the species' diet elsewhere in their invasive range (Kornis et al. 2012). Sensitivity analysis (see supplementary materials S4) also suggests that these may contribute to their diet, if the Guldborgsund population does feed on these prey taxa. Nonetheless, for the purposes of this study, these results support that this population appears to feed from a variety of prey taxa, and there is significant among-individual variation in isotope values.

There were surprisingly few links between trophic values and state variables, although there was some evidence that trophic level was related to fish health. Ontogenic morphological changes are linked to feeding shifts in this species (Andraso et al. 2011), where smaller prey items like zooplankton and insect larvae make up large proportion of juvenile diets, while larger hard-bodied prey items become more significant in adult diets (Kornis et al. 2012; Borcherding et al. 2013; Oesterwind et al. 2017). Although this can produce ontogenic shifts in trophic level, such as increasing $\delta^{15}N$ in larger individuals, this appears to be dependent on the specific population (Brush et al. 2012; Herlevi et al. 2018). For example, if preferred adult prey items are not necessarily higher in $\delta^{15}N$ than juvenile prey items, no shift may be observable. Additionally, our experimental group had limited size variation by design. No direct effects of condition were found, while males appeared to feed at a higher trophic level than the few females in the study. In addition, supplementary analysis identified that survivorship in the experimental cohort favoured larger, male fish that had slightly higher $\delta^{15}N$ scores (see supplementary materials S2). This may indicate a relationship between fish health and trophic values, for example females both performed more poorly in the lab and appeared to feed at a lower trophic level. This may however also be a biproduct of the experimental conditions, where larger and potentially more competitive/aggressive males may better adapt to the relatively high-density laboratory housing conditions. Nonetheless, this suggests that intraspecific state differences may have a role in trophic differentiation within this population, but these were not captured by our set of state variables. Studies with larger sex-balanced cohorts should be better able to disentangle potential sex of condition effects on trophic interactions.

Linking personality with trophic variation in wild populations is time and resource intensive due to the challenges of community-wide sampling for food-web analysis alongside repeated-measures behavioural experiments. This study shows this is viable, when using a species that has relatively well-developed literature to inform SIA mixed modelling/diet analysis, and that performs well with individual PIT tagging/tissue sampling. This builds on a small growing set of studies using laboratory-based behavioural measures combined with SIA to measure trophic variation in the wild (e.g., Glon et al. 2016; Adey and Larson 2020; Theódórsson and Ólafsdóttir 2022). A benefit of this approach (cf. studies using tracking or similar measures of behaviour in the wild; e.g., Monk et al. 2023) is to allow among-individual behavioural variation to be quantified independently of extrinsic environmental variation, focusing on intrinsic state-dependent variation and variation associated with underlying genetic/epigenetic variation. This also allows results to be assessed in relation to a large body of animal personality experimental research conducted over the last 20 years.

Nonetheless, the poor survivorship and strong sex biases in the experimental population also limit the power of our analyses and highlight the need for refinement. For example, a correlation was found between $\delta^{13}C$ and boldness (i.e., as centre area use) using the average score of individuals across trials, but this effect was only marginally significant and was not detected in trial 1 scores alone (i.e., which had a larger sample size, and may be less impacted by survivorship bias). This correlation points to personality as a factor that can influence trophic variation within populations, but correlations may also be a produced by personality-biased survival in the cohort so this relationship should be interpreted very cautiously (e.g., Bell and Sih 2007). This may be improved by optimising sampling to periods where gobies tend to be in better condition, as sampling here was directly impacted/delayed by Covid-19 restrictions and occurred in summer where Baltic Sea round goby populations tend to be in relatively poorer condition (Brauer et al. 2020). Notably, severe declines in round goby abundance were observed throughout the Baltic region around 2020 (see for example Kruze et al. 2023), which may also suggest an external environmental factor may have influenced the high mortality observed in this cohort. Importantly, procedures used for tissue sampling and tagging are very low impact, and do not appear to be a factor that influenced the health of fish in the laboratory (see supplementary materials S2).

Studies disentangling personality and state effects on ecological interactions will be important for understanding the impacts of invasive species on recipient ecosystems. Behavioural and trophic variation appears to play an important role in the round goby’s invasion process, both in their ongoing spread through the Baltic region, and in the post-arrival/establishment phases where populations can reach extremely high densities and have acute impacts on the composition of benthic communities through predation. Further studies will provide greater insights into the round goby’s impacts, by using refined sampling approaches to address limitations of this study, and by assessing intraspecific variation across multiple populations across their invasion front at different stages of establishment.

Data availability statement

All data and code used for processing, analysis and visualization are available at Open Science Framework (https://osf.io/rnz7q/, doi: 10.17605/OSF.IO/RNZ7Q).

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Author contributions (CRediT taxonomy)

NPM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

JWB: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Supporting information

S1. Sampling technical information

S2. Sensitivity analysis – Survivorship bias

S3. Prey groupings for SIA and diet reconstruction

S4. Sensitivity analysis – SIA input parameters

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Tables

Table 1: Behaviour variable descriptions, distributions/transformations, and estimates of raw and adjusted repeatability (i.e., adjusted to account for experimental grouping factors, ArenaID, TankID, TrialRound and TrialDay).

Assay	**Variable** *(raw unit, name)*	**Description**	**Distribution**	**Repeatability**	
				Raw	**Adjusted**
Activity	Distance *(mm, dist)*	Total distance moved over the full 20 min trial period.	Gaussian	0.47^{***} [0.28, 0.63] (P < 0.001)	0.53^{***} [0.3, 0.73] (P < 0.001)
	Average speed *(mm/s, avespeed_mob)*	Average speed during the trial, excluding periods not actively moving (i.e., excluding motion <5 mm/s)	Gaussian	0.24^{**} [0.02, 0.43] (P = 0.0097)	0.34^{***} [0.12, 0.52] (P < 0.001)
	Time frozen *(s,timefrozen_tot)*	Total time spent frozen. A fish is considered frozen where they are immobile (i.e., speed < 5mm/s), and move a total distance < 25 mm over a period >3 s..	Gaussian (*ln(x) transformed*)	0.45^{***} [0.23, 0.65] (P < 0.001)	0.5^{***} [0.27, 0.69] (P < 0.001)
	Centre use *(mm, centrescore)*	Score of the average distance from the edge of the arena, calculated using the time fish spent in each edge zone (0 – 2.5, 2.5 – 5.0 cm, etc., see Fig. 2A).	Gaussian	0.39^{***} [0.15, 0.54] (P < 0.001)	0.37^{***} [0.16, 0.54] (P < 0.001)
Exploration	Emergence latency *(s, emergelat)*	Time to emerge from a refuge after the barrier is removed (see Fig. 2B). Maximum latency is 2700 s (45 mins).	Binary (*1 = latency < 50s, 0 = latency ≥ 50s*)	0.71^{***} [0.36, 0.86] (P < 0.001)	0.71^{***} [0.34, 0.83] (P < 0.001)
	Endpoint latency *(s, endpointlat)*	Time to reach the end of the arena after the barrier is removed (see Fig. 2B). Maximum latency is 2700 s (45 mins).	Binary (*1, latency ≤ 160s; 0, latency > 160s*)	0.58^{***} [0.18, 0.74] (P < 0.001)	0.58^{***} [0.22, 0.78] (P < 0.001)

Table 2. Effects of state variables (i.e., TL, sex and condition) and habituation (i.e., replicate) on behaviour in *Activity* and *Exploration* assays. Fixed effects with significantly non-zero estimates are in bold. Positive effects on distance and average speed show increased activity, while positive effects on time frozen show reduced activity. Positive effects on centre use show greater use of central/exposed areas. Conditional R^2 (R^2_{Con}) represents the proportion of total variance explained by fixed and random effect parameters, and marginal R^2 (R^2_{Mar}) represents the proportion represented by fixed effects alone.

Model						$\mathbf{R^2_{Con}}$	$\mathbf{R^2_{Mar}}$
-factors	*Estimate [95% CI]*	*S.E.*	*df*	*t/z*	*P*		
Distance$_{\textit{(dist, gaussian lmer model)}}$						0.754	0.302
-intercept	21047.33 [11430.63, 30659.18]	5026.15	42.14	4.19	< 0.001		
-Sex$_{\mathbf{male}}$	**18636.59 [8516.87, 28785.1]**	**5287.78**	**38.96**	**3.52**	**0.001** **		
-TL$_{\textit{Z-scaled}}$	1939.41 [-1463.95, 5351.39]	1777.78	39.3	1.09	0.282		
-CondManual$_{\textit{Z-scaled}}$	3327.89 [-95.4, 6758.82]	1788.18	38.62	1.86	0.07		
-TrialDay.C$_{\mathbf{Z\text{-}scaled}}$	**-6116.69 [-7628.35, -4600.69]**	**766.76**	**69.9**	**-7.98**	**< 0.001** ***		
Ave. speed$_{\textit{(avespeed\_mob, gaussian lmer model)}}$						0.605	0.322
-intercept	34.7 [28.57, 40.83]	3.21	41.54	10.81	< 0.001 ***		
-Sex$_{\mathbf{male}}$	**11.77 [5.14, 18.39]**	**3.47**	**40.81**	**3.39**	**0.002** **		
-TL$_{\textit{Z-scaled}}$	-0.29 [-2.51, 1.93]	1.16	40.26	-0.25	0.804		
-CondManual$_{\textit{Z-scaled}}$	1.23 [-1.01, 3.46]	1.17	39.75	1.05	0.3		
-TrialDay.C$_{\mathbf{Z\text{-}scaled}}$	**-5.51 [-6.84, -4.17]**	**0.68**	**78.03**	**-8.12**	**< 0.001** ***		
Time frozen$_{\textit{(ln(timefrozen\_tot) , gaussian lmer model)}}$						0.774	0.326
-intercept	6.61 [6.03, 7.18]	0.3	43.11	22.15	< 0.001 ***		
-Sex$_{\mathbf{male}}$	**-1.18 [-1.76, -0.58]**	**0.31**	**38.23**	**-3.86**	**< 0.001** ***		
-TL$_{\textit{Z-scaled}}$	-0.04 [-0.24, 0.15]	0.1	38.4	-0.41	0.682		
-CondManual$_{\mathbf{Z\text{-}scaled}}$	**-0.28 [-0.48, -0.08]**	**0.1**	**37.93**	**-2.72**	**0.01** **		
-TrialDay.C$_{\mathbf{Z\text{-}scaled}}$	**0.38 [0.28, 0.5]**	**0.05**	**8.76**	**7.07**	**< 0.001** ***		
Centre use$_{\textit{(centrescore, gaussian lmer model)}}$						0.475	0.088
-intercept	3.59 [3, 4.17]	0.31	42.33	11.73	< 0.001 ***		
-Sex$_{\textit{male}}$	0.32 [-0.29, 0.93]	0.32	39.25	1.01	0.318		
-TL$_{\mathbf{Z\text{-}scaled}}$	**0.23 [0.03, 0.44]**	**0.11**	**38.93**	**2.18**	**0.035** *		
-CondManual$_{\textit{Z-scaled}}$	-0.03 [-0.24, 0.17]	0.11	38.33	-0.28	0.777		
-TrialDay.C$_{\textit{Z-scaled}}$	0.05 [-0.08, 0.17]	0.07	72.01	0.71	0.48		
Emergence$_{\textit{(emergelat, binary glmer model)}}$						0.762	0.028
-intercept	-1.8 [-5.37, 1.78]	1.82	NA	-0.99	0.324		
-Sex$_{\textit{male}}$	1.89 [-1.93, 5.71]	1.95	NA	0.97	0.333		
-TL$_{\textit{Z-scaled}}$	0.13 [-1.12, 1.38]	0.64	NA	0.21	0.835		
-CondManual$_{\textit{Z-scaled}}$	0.26 [-1, 1.52]	0.64	NA	0.41	0.684		
-TrialDay.C$_{\textit{Z-scaled}}$	0.06 [-0.55, 0.66]	0.31	NA	0.19	0.852		
Endpoint$_{\textit{(endpointlat, binary glmer model)}}$						0.633	0.036
-intercept	-1.49 [-4.27, 1.29]	1.42	NA	-1.05	0.292		
-Sex$_{\textit{male}}$	1.61 [-1.35, 4.58]	1.51	NA	1.07	0.286		
-TL$_{\textit{Z-scaled}}$	-0.31 [-1.25, 0.64]	0.48	NA	-0.63	0.527		
-CondManual$_{\textit{Z-scaled}}$	0.08 [-0.86, 1.01]	0.48	NA	0.16	0.871		
-TrialDay.C$_{\textit{Z-scaled}}$	-0.12 [-0.67, 0.42]	0.28	NA	-0.44	0.658		

Table 3. Spearman's correlations between behavioral variables, including the correlation coefficient estimate (r_s) and p-value.

	Distance *(dist)*	Ave. speed *(avespeed_mob)*	Time frozen *(timefrozen_tot)*	Centre use *(centrescore)*	Emergence *(emergelat)*	Endpoint *(endpointlat)*
Distance *(dist)*		**0.814** **(P < 0.001)** ***	**-0.914** **(P < 0.001)** ***	0.067 (P = 0.473)	**-0.426** **(P < 0.001)** ***	**-0.381** **(P < 0.001)** ***
Ave. speed *(avespeed_mob)*			**-0.612** **(P < 0.001)** ***	0.165 (P = 0.074)	**-0.291** **(P = 0.002)** **	**-0.22** **(P = 0.02)** *
Time frozen *(timefrozen_tot)*				0.009 (P = 0.925)	**0.483** **(P < 0.001)** ***	**0.459** **(P < 0.001)** ***
Centre use *(centrescore)*					-0.175 (P = 0.066)	-0.105 (P = 0.275)
Emergence *(emergelat)*						**0.9** **(P < 0.001)** ***
Endpoint *(endpointlat)*						

Table 4. Spearman's correlations between behavioral and trophic variables, including the correlation coefficient estimate (r_s) and p-value.

	$\delta^{13}C$		**$\delta^{15}N$**	
	Trial 1 score	Mean score across trials	Trial 1 score	Mean score across trials
Distance *(dist)*	-0.205 (P = 0.192)	-0.111 (P = 0.538)	0.168 (P = 0.287)	0.02 (P = 0.911)
Ave. speed *(avespeed_mob)*	-0.216 (P = 0.169)	-0.18 (P = 0.314)	0.134 (P = 0.398)	0.041 (P = 0.819)
Time frozen *(timefrozen_tot)*	0.121 (P = 0.446)	-0.054 (P = 0.766)	-0.196 (P = 0.213)	-0.068 (P = 0.705)
Centre use *(centrescore)*	-0.283 (P = 0.069)	**-0.361** **(P = 0.040) ***	-0.106 (P = 0.504)	-0.131 (P = 0.467)
Emergence *(emergelat)*	0.08 (P = 0.614)	-0.08 (P = 0.667)	-0.185 (P = 0.241)	0.011 (P = 0.954)
Endpoint *(endpointlat)*	0.082 (P = 0.605)	-0.025 (P = 0.895)	-0.141 (P = 0.374)	0.016 (P = 0.932)

Figure legends

Figure 1. Sampling occurred over a 2 ha area in the Guldborgsund strait (pink diamond). The site is in the western Baltic Sea, in south-eastern Denmark, where round gobies were first detected ca. 2009.

Figure 2. Experimental designs for the (A) *Activity*, and (B) *Exploration* assays, and (insets) the arrangement of each arena type when running multiple assays concurrently. The *Activity* arena is an open opaque white box with 10 cm water height. For tracking, the arena is divided into five zones measured from its edge [i.e., **(i),** 0 – 2.5, 2.5 – 5.0, 5.0 – 7.5, 7.5 – 10.0, >10.0 cm], that are used to calculate centre use. The *Exploration* arena is an opaque black lane lined with a thin base layer of sand. An enclosed refuge [i.e., **(ii)**] is at one end, where the individual fish are held during acclimation, before a barrier separating the refuge from the arena is lifted to initiate the trial. An individual is taken to have emerged into the arena once >50 % of its body length has moved out of the refuge. The fish is then taken to have reached the endpoint of the arena once >50 % of its body length has entered the final 16 x 16 cm 'end-zone' section [i.e., **(iii)**].

Figure 3. SIA biplot of δ^{13}C and δ^{15}N values for the Guldborgsund community. Each circle represents one individual fish (grey) or taxonomic grouping (other colours). Error bars represent ± 1 SD. Circles respresenting taxonomic groupings are labelled by their ID,s and coloured according to their class classification: Bivalvia (bright blue – *Cerastoderma glaucum* [V01], *Mytilus edulis* [V02], *Mya arenaria* [V03]); Gastropoda (purple – *Theodoxus fluviatilis* [V04], Littorinimorpha spp. [V05]); Malacostraca (chartreuse – *Crangon crangon* [V06], Palaemon spp. [V07], Amphipoda spp. [V08], Idotea spp. [V09]); Ostracoda (red – Podocopida spp. [V11]); Insecta (brown – Diptera spp. [V12]); Polychaeta (orange – *Arenicola marina* [V15], Polychaeta spp. [V16]); and, *Actinopterygii* (light blue – *Pungitius pungitius* [V18], *Gasterosteus aculeatus* [V19], Syngnathidae spp. [V20], Gobiidae spp. [V21], and *N. melanostomus* < 8 cm TL [V22]). Primary producers are included as blocks, which represent the range of ± 1 SD from the mean for each source, which include macroalgae (S01 – S04, e.g., bladder wrack, filamentous algae, etc.), riparian/terrestrial vegetation (S05 – S06), and phytoplankton/FPOM (S08). There was insufficient biomass for replicates of one individual (G48), so error bars were not included.

Figure 4. MixSIAR posterior density distributions of diet component estimates for the Guldborgsund round goby population. Distributions represent the estimated proportion of their diet that may be associated with each component.

Figures

Figure 1

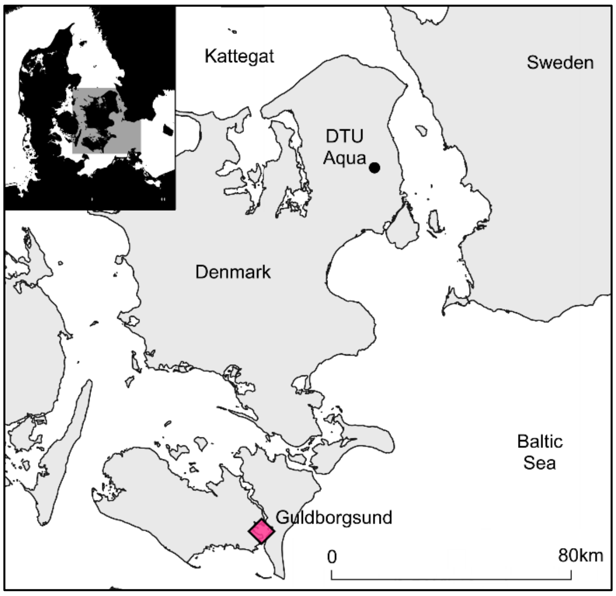


Figure 2

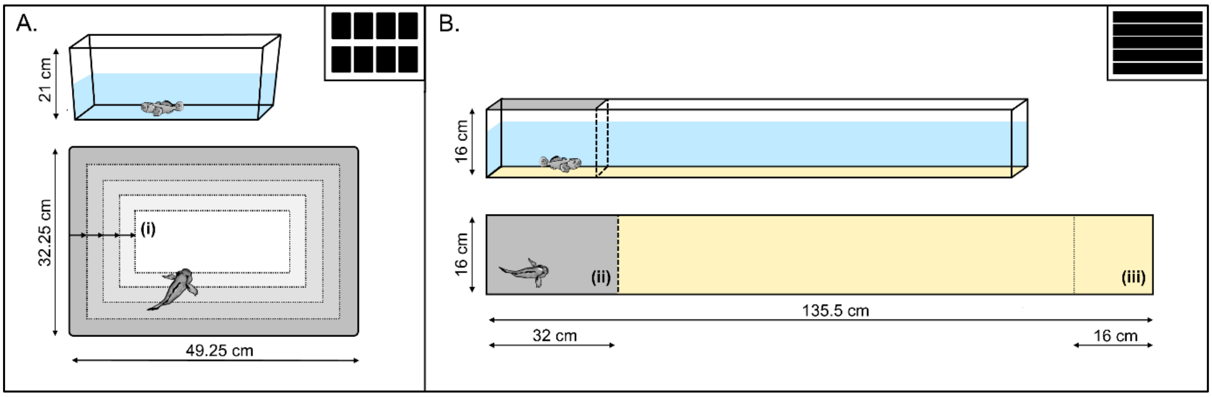
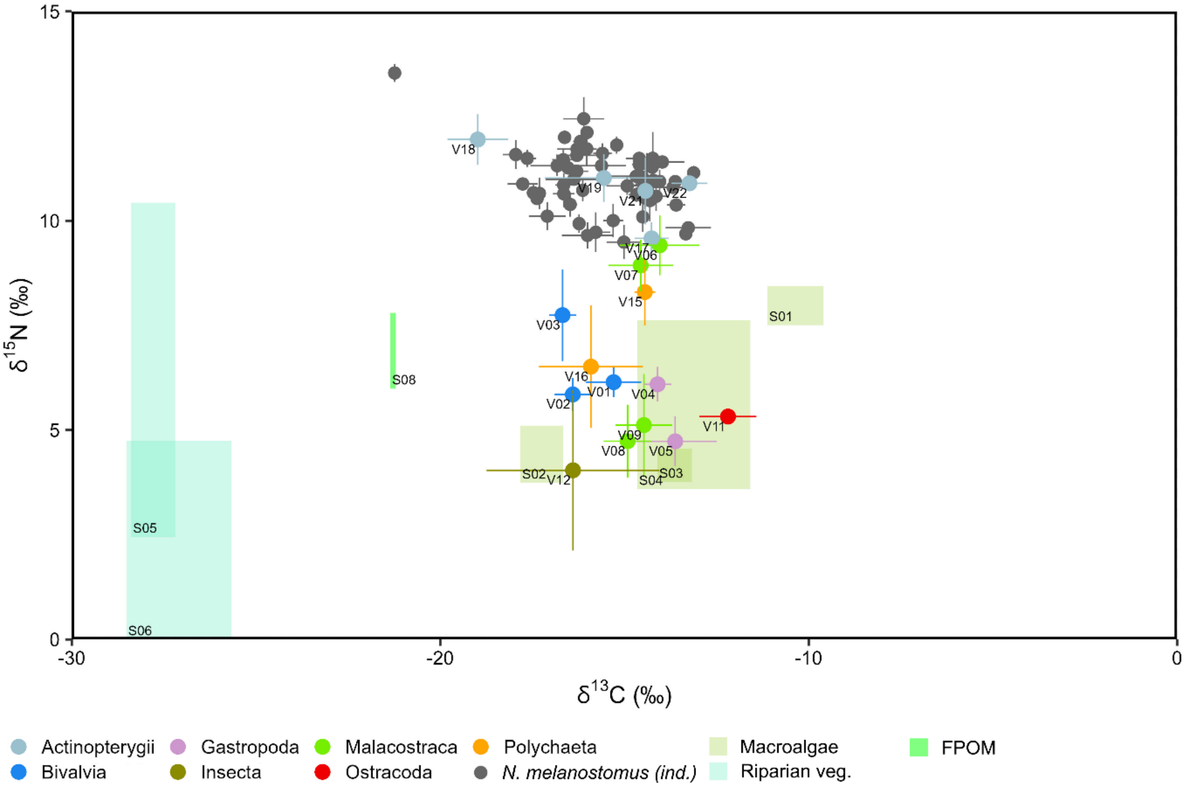


Figure 3


15
10
5
0
-30
-20
-10
0
$\delta^{15}N$ (‰)
$\delta^{13}C$ (‰)
V18
V19
V21
V22
V17
V06
V07
V15
V03
V16
V01
V02
V04
V11
V09
V08
V05
S02
V12
S04
S03
S01
S08
S05
S06
Actinopterygii
Bivalvia
Gastropoda
Insecta
Malacostraca
Ostracoda
Polychaeta
N. melanostomus (ind.)
Macroalgae
Riparian veg.
FPOM

1041 *Figure 4*