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

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**Short Running Title:** Individual behavioural and trophic variation

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## Abstract

An individual animal’s behavioural traits can influence the outcome of ecological interactions within their food-web, including what they eat, how vulnerable they are to predation and who they compete with. Despite this, few studies have directly measured links between behavioural and trophic variation in wild populations. For invasive species such as the round goby (*Neogobius melanostomus*), populations in invaded ecosystems are often found to have strong among-individual variation in behavioural traits (e.g. bold-exploratory traits) that can differ between populations across their invasion front. Therefore an individualized approach to invasive populations and their ecological interaction may be valuable to understanding how invasive populations impact recipient ecosystems. By using non-lethal methods to measure food web variation (e.g. stable isotope analysis using fin clips) and individual tagging (e.g. passive integrated transponder tags), we analysed behavioural and trophic variation concurrently to test if behavioural variation is directly linked to food-web variation in the wild. Focusing on an established high-density invasive population of round gobies in Guldborgsund sound in the southwest Baltic Sea, we found stable among-individual behavioural variation in this population in activity, boldness and exploration traits in novel environment and refuge emergence assays. We also found among-individual isotopic variation, with particularly high variation in carbon-12 – carbon-13 (δ13C) values showing that round gobies differ in what are feeding on and/or where they forage, indicating among-individual variation in how they impact benthic fauna/prey communities. There were limited links between behavioural and trophic variation, nonetheless study shows that measuring behavioural-trophic correlations is a viable approach for exploring the role of behavioural traits in individual-level ecological variation in the wild.

**Keywords** individualized niche, invasion, personality, boldness, exploration, Gobiidae

## Introduction

Intraspecific behavioural variation is closely linked to many ecological processes, including predator-prey dynamics, intraspecific/social interactions, and biological invasions (Réale et al., 2007; Wolf and Weissing, 2012). Behavioural differences among individuals may be derived from a combination of underlying genetic/epigenetic variation and phenotypic plasticity (Nussey et al., 2007; Dingemanse et al., 2010), and are commonly expressed across a wide range of taxa including serious invasive pests 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). This variation may play an important role in the success or failure of invasions at multiple stages, including the transport/introduction and establishment of new invasive populations (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) may drive range expansion at invasion fronts (Cote et al., 2010; Thorlacius et al., 2015; Rehage et al., 2016). This trait-biased process may then contribute to phenotypic divergence between populations along an invasion gradient in an ‘invasion succession’ (Gruber et al., 2017; Thorlacius and Brodin, 2018). Therefore, behavioural variation may be an important consideration when studying the impacts of invasive animals on invaded ecosystems, and how this may change following establishment.

The composition of behavioural traits within a population can have a strong influence on how that population in interacts with its local community, e.g. via predator-prey and competitive interactions (Moran et al., 2017).

Behavioural decisions and the individualised niche

Among-individual variation in risk-taking behaviour is common, where the term ‘risk’ is often used in relation to an individual’s willingness to engage in behaviour involving novelty (e.g. engaging or interacting with a novel environment or object, White et al., 2013) or direct or indirect predation risk (Réale et al., 2007). Often referred to as boldness or exploratory traits, engaging in risky behaviours often involves a trade-off between resource acquisition and potential mortality/predation (Moran et al., 2020). Intraspecific behavioural variation can influence the strength and outcome of ecological interactions , and in risk-taking has been linked differences in feeding behaviour and vulnerability to predation in some specific cases (Jolles et al., 2013, 2016; White et al., 2013). Therefore, quantifying links between behavioural variation and ecological interactions may be critical to understanding how an individual and their population affect their environment.

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Where behavioural traits are linked to feeding interactions, the traits of a predator population can influence the composition of their prey communities, with potentially cascading effects across trophic levels. For an invasive species though, among-individual variation may determine the nature of their impacts on their invaded ecosystems (Juette et al., 2014).

A challenge to analysing among-individual level and behavioural variation simultaneously is the need to conduct minor, although invasive procedures such as gastric lavage for gut content analysis, or tissue sampling for isotope analysis. Stable isotope analysis is tool that allows for diet reconstruction and the description of trophic relationships (Post, 2002; Boecklen et al., 2011). As organic tissue is consumed and assimilated into the tissue of higher level consumers/predator, the ratios of carbon-12 – carbon-13 (hereafter ‘δ13C’) and nitrogen-14 – nitrogen-15 (hereafter ‘δ15N’) 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 relationships between consumers and their prey, and infer links between consumers and primary carbon producers (e.g. phytoplankton, macro-algaes, aquatic vegetation etc.) (Layman et al., 2012). Assimilation rates may vary, but isotope ratios of softer tissues such as skin/scales, muscle and fins can be used to infer diet variation over periods of several weeks to months (Thomas and Crowther, 2015; Britton and Busst, 2018). Fin tissue are particularly useful for non-lethally measuring to isotopic variation in fish, providing that one accounts for potential within-fin variation (Jardine et al., 2011; Hayden et al., 2015). Samples can be taken with relatively little stress to the fish, and minimal tissue is required for isotopic analysis (e.g. 0.5 mg or less of dry tissue), so may be used to measure to amount of trophic variation in a population while maintaining that population for further analysis.

Measuring trophic variation is particularly useful for invasive species, such as the round goby ( to describe the interactions between invasive populations and the food webs they have invaded.

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<https://onlinelibrary.wiley.com/doi/full/10.1111/eff.12472>

<https://www.nrcresearchpress.com/doi/full/10.1139/cjz-2014-0127> Best practices for use of stable isotope mixing models in food-web studies

Few studies have directly measured how among-individual behavioural trait variation (i.e. activity, bold-exploratory traits etc.) translates to ecological variation in wild populations, which may be especially important for understanding how invasive animal populations impact the communities that they invade. Therefore, the overarching objective of this study is to establish an approach that can concurrently measure both individual behavioural and trophic state variation in animals collected from wild populations. Specifically, we sought to:

1. Quantify among-individual variation in bold-exploratory behavioural traits and the trophic state within a well-established invasive population of round gobies. As among-individual behavioural variation is common in animals [REFs], including the round goby [REFs], we expected to find among-individual variation in behavioural variables taken from bold-exploratory assays (i.e. activity, edge use, emergence-exploration), as well as in trophic state variables (i.e. in δ13C and δ15N values). We also explored to role of state differences, e.g. body size, body condition and sex, as a source of behavioural and trophic variation in these populations.
2. Explore whether individual behavioural variation may be having an influence the round goby’s interactions with prey species. First, we calculated correlations between individual isotopic values and round personality traits (i.e. those that show among-individual variation), to identify if any specific traits area linked to their trophic interactions. Second, we use stable isotopic analysis (‘SIA’) of round goby prey species and mixing models to quantify the relative contributions of prey taxa to round goby diets.

## Methods

### Field Sampling

Round gobies and their potential prey items were sampled from a shallow brackish estuary (Guldborgsund, 54.69645°, 11.84067°, from 16-17th June 2020; Fig. 1). Guldborgsund was one of the first Danish marine areas to be invaded by the round goby, first being observed ca. 2009. Therefore it can be considered a relatively well-established self-sustaining population, which are characterised by high population densities where round gobies are likely to experience strong 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 both the abundance of their preferred prey items (e.g. gastropods and bivavles; van Deurs et al., 2021), and because they show can high site affinity around rocky structures as a source of shelter, food and nesting sites (Lynch and Mensinger, 2012; Christoffersen et al., 2019). Round gobies are most active in coastal and inlet areas over the Spring-Summer breeding period, and particularly from April to June in the south-eastern Baltic region (Marentette et al., 2011; Brauer et al., 2020). 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 will be primarily linked to their local diet within the estuary.

Round gobies were collected using a combination of fyke nets, and baited box and cylinder traps, which were deployed for 24 hours in eight points evenly spaced across the sampling area (for further details see supplementary materials S1). Multiple net types methods were used to minimise personality biased sampling (Biro and Dingemanse, 2009; Michelangeli et al., 2016), and active sampling via push nets and large dip nets was also attempted unsuccessfully. Nonetheless passive sampling alone has previously performed well at capturing unbiased samples in round gobies (e.g. Thorlacius et al., 2015). Fish below 80 mm total length (TL) were excluded, as round gobies below this size may have not developed the adult morphological features required for feeding on hardbodied prey items, which make up a large part of their adult diet (Andraso et al., 2011). So that any trophic variation observed here is unlikely to be linked to this ontogenetic transition.

Fish (n = 55) were transported to DTU Aqua fish stable facility (Fig 1, Lyngby, Denmark) and maintained under in a 12:12 hr light:dark cycle, at 10 ± 1 °C and 16 ± 1 ppt salinity and fed to satiation three times per week with commercial high-nutrition pellet fish feed (3mm Ivory Ex composite pellets, Aller Aqua, Denmark). All holding tanks were linked to a recirculating physical and biological filtration system, which maintained standardised physical conditions across tanks and a constant gentle flow within tanks. The salinity used is within the natural range of the source location (Feistel et al., 2010), and is well within the species’ osmoregulatory tolerance (Behrens et al., 2017; Puntila-Dodd et al., 2021).

Prey fauna were collected using a combination of methods to ensure a cross-section of the mobile and sessile fauna community were represented. Sampling included: HAPS handheld cores (sampled area: 0.0143 m2, depth 28 cm); push net samples (net width 65 cm, mesh size 1 cm, 5m transects); quadrat samples (hand/paint scraper collections of all benthic fauna within 50 x 50 cm quadrats); and baited box/cylinder traps (as described above). These were chosen to so that sampling would capture a comprehensive cross-section of local benthic infauna, sessile fauna and mobile fauna. Samples were rinsed through a 0.5 mm sieve and rinsed with deionised (DI) water. Eight replicates of each sample type was collected within the sampling area, and were placed on ice for transport (approx. 2 hrs) then frozen at -40 °C before further processing.

Samples of potential primary carbon producers were also collected provide additional context to any observed isotopic variation in round goby and prey samples. This included three replicate samples were collected by hand of any dominant algae types. Coarse particular organic matter (POM) water taken from core and quadrat samples, which was primarily from woody or leafy terrestrial/riparian vegetation. To represent the phytoplankton community, three replicate samples were taken of fine particular organic matter (FPOM) using water collected from the deepest area of the sampling area (depth 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), so represent a 0.7 – 47 µm FPOM fraction that appears to successfully capture the local phytoplankton community in late Spring-early Summer. Samples and filters were then frozen at -40°C before further processing.

### Individual tagging and tissue sampling

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). Round gobies cope well with internal tagging, and PIT tagging is not expected to affect survival and growth (Ruetz et al., 2006; Cookingham and Ruetz III, 2008). Caudal fin tissue samples were then taken using surgical scissors and stored individually at -40°C before analysis.

The extreme outer edge (<5 mm) of fins was sampled to standardise sampling between individuals, which were separated into three replicate samples per individual to account for within-fin variation (Hayden et al., 2015; Britton and Busst, 2018). Although neither tagging nor tissue sampling is expected to influence of behaviour, the combination of both may cause additional stress. Therefore we conducted an additional pilot experiment, but found no effects of tissue sampling and/or fin clips on round goby activity or bold-exploratory behaviours after 10 days post-tagging (see supplementary materials S2). Fish were had their total length (‘TL’) measured before tissue sampling and tagging, and were inspected for eye fluke parasites that may influence behavioural responses in this species (Flink et al., 2017). The full process took under 60 s per fish, and fish were weighed and sexed several days later to avoid further stressing fish during this procedure.

### Behavioural analysis

Two forms of behavioural assay were used (*Activity* and *Exploration*), each repeated three times at one-week intervals. Before and between behavioural trials, fish were distributed between three smaller round 800L (1 x 1 m; height x diameter, filled depth ~ 0.8 m) holding tanks enriched with artificial eel grass. Of the 55 fish returned to the lab, the majority (33, 60%) survived until the full 30 day experimental period from sampling. Given this attrition rate is relatively high, the impact of this our results has been assessed via sensitivity analysis (see supplementary materials S3). Prior to behavioural experiments starting some fish were moved between holding tanks to maintain similar densities, whereas during the experiments fish were not moved to avoid altering their social environment. Nonetheless, fish densities stayed relatively constant between tanks until to end of the experimental period.

The *Activity* assay used a standard open field design to measure activity and boldness-exploratory (or risk-taking) traits in a novel environment (Fig 2A) (Toms et al., 2010). Individual fish were selected blindly using dip nets from housing tanks and placed into a novel arena using smaller plastic transport tubs (24 x 34 cm) to minimise stress by ensuring that fish remain entirely in water as they are moved into the novel arena. Eight identical arenas were used, which are identical opaque white PET plastic boxes (internal dimensions at its base = 32.25 x 49.25 cm, height = 21 cm, filled to 10 cm depth). Arenas were arranged in a 2 x 4 grid under a laboratory fluorescent light in an area separated by a curtain, so arenas had consistent light conditions (~ 45 lux) and external sound/light effects were minimised. Behaviour is recorded for 20 minutes using modified web camera was positioned centrally 2 m above the arenas (Logitech BRIO 4K Ultra HD webcam, Logitech, Switzerland), following a 5 minute acclimation period that is intended to limit any acute effects on behaviour due to their netting from holding tanks and introduction into the arenas. To avoid any carryover effects between trial rounds, arenas are emptied, rinsed with deionised water and filled with new filtered experimental water following each trial.

Fish movement in the *Activity* assay was tracked using Toxtrac v2.90 (Rodriguez et al., 2018). To manually calibrate each arena for tracking, the pixel/mm ratios for each arena were calculated using ImageJ v1.52a (Schneider et al., 2012). Variables were extracted relating to activity levels and edge use in the arena (see Table 1), where more activity in a novel environment and greater use of more exposed central areas is considered higher-risk or exploratory/bold behaviour (Moran et al., 2016, 2021).

The *Exploration* assay used a refuge emergence design to measure an individual’s willingness to enter and explore an novel environment (Fig 2B) (Toms et al., 2010). Individual fish were introduced into an (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 from a position behind the refuges, so that the observer was not visible to the fish. Behaviour was then filmed as above for 45 minutes, with the camera positioned centrally 1.5 m above the arenas. *Exploration* arenas are connected to an inflow from the laboratories recirculation system to maintain constant water quality throughout the experiment (approximately 5.5 mL/s inflow from the refuge end; note, this was not required in *Activity* arenas). Arenas were completed flushed with filtered water between trials to avoid carryover effects.

Behaviour was manually scored from videos, focusing on their tendency to emerge and explore to the endpoint of the arena (Table 1). Fish are faster to emerge and/or explore a novel environment are generally considered higher-risk or more exploratory/bold behaviours (Moran et al., 2016, 2021). Fish were fed on the day before each trial to ensure satiation levels were consistent. After each assay, fish had their PIT tag number recorded to confirm their individual identity and were checked for any physical injuries or lethargy that may have influenced their behaviour in trials. Both assays were repeated three times at one week intervals, with *Exploration* assays run two days after *Activity* assays to limit the influence residual carryover effects between trials (Bell, 2013).

### Stable isotope processing and analysis

Unless otherwise stated, isotope samples were prepared following standard SIA methods for marine aquatic food webs (Jardine et al., 2003). Fish fin samples were thoroughly rinsed with purified DI water to remove any surface contamination. The outer 5mm of the fin are relatively new growth and tend to have low levels of parasitic infection, nonetheless samples were each inspected under a dissecting microscope and any sections with visible parasite nodules/fungal spots were removed.

Prey items were picked out of bulk samples under magnification, thoroughly rinsed and sorted into taxonomic groupings to at least Family or Order level where possible (see specific groupings in see supplementary materials S3). Soft tissue from gastropods was removed from their shell, as carbon-based precipitates reflect the isotopic ratios of the inorganic environment whereas their soft tissue reflects their diet (Post, 2002). Guts were dissected out where possible and soft tissue 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 replicates per taxa were produced using tissue from distinct individuals, with animals used in replicates taken from separate bulk samples/parts of the sampling area where possible. Three replicates of major primary producers were rinsed and any invertebrates removed, including macroalgaes (e.g. bladder wrack, filamentous algae) and terrestrial/riparian POM (e.g. *phragmites detritus*, woody and leafy detritus).

Fish fin, prey taxa 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 for analysis and replicate samples per individual are able to account for and estimate within-fin/residual variation. All other samples (excluding filters) were homogenized by grinding into a fine powder. Fin and prey taxa 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 δ13C and δ15N 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 δ13C and δ15N are calculated by δ13C/δ15N = (((R measured/R reference)-1) x 1000), and are expressed in per mille (‰) relative to the isotopic ratio of Vienna Pee Dee Belemnite standard (RVPDB = 0.0111797) for carbon and atmospheric N2 (RAir = 0.0036765) for nitrogen.

### Statistical analysis

Behavioural repeatability

- fish excluded from analysis where injuries were found post-trial inspections,

- Physical variables (e.g. TL, condition and sex were included a predicator variables based on their values collected during tagging and 1-week post tagging. .

Discrimination factor (see supplementary materials S4)

## Results

Trophic variation for round gobies was higher in magnitude for δ13C than δ15N, suggesting that differential resource is the primary source of variation within the population.

## Discussion

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## Author contributions (CRediT taxonomy)

## Data Availability Statement

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

## Supporting Information

S1. Sampling gear and effort

## S2. Tagging and tissue sampling effects on behaviour

## S3. Sensitivity analyses – Survivorship bias

S4. Prey groupings

S5. Sensitivity Analysis–Isotopic discrimination factors

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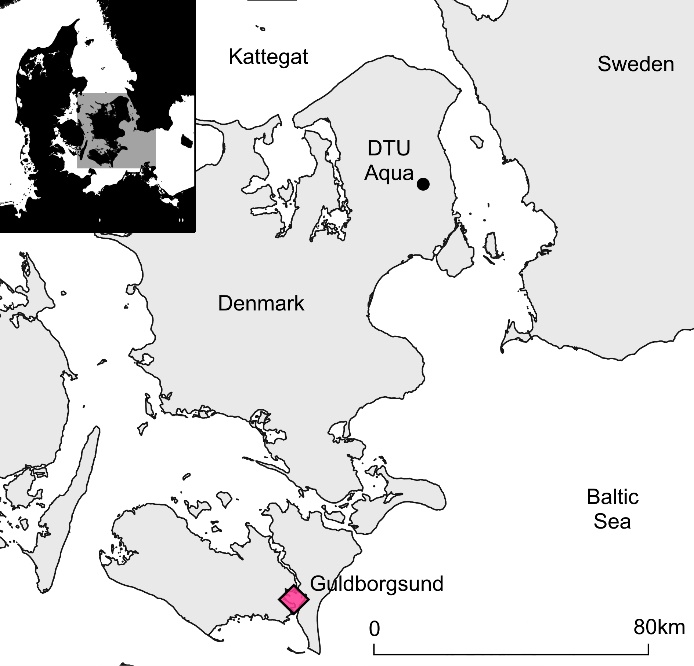
**Tables**

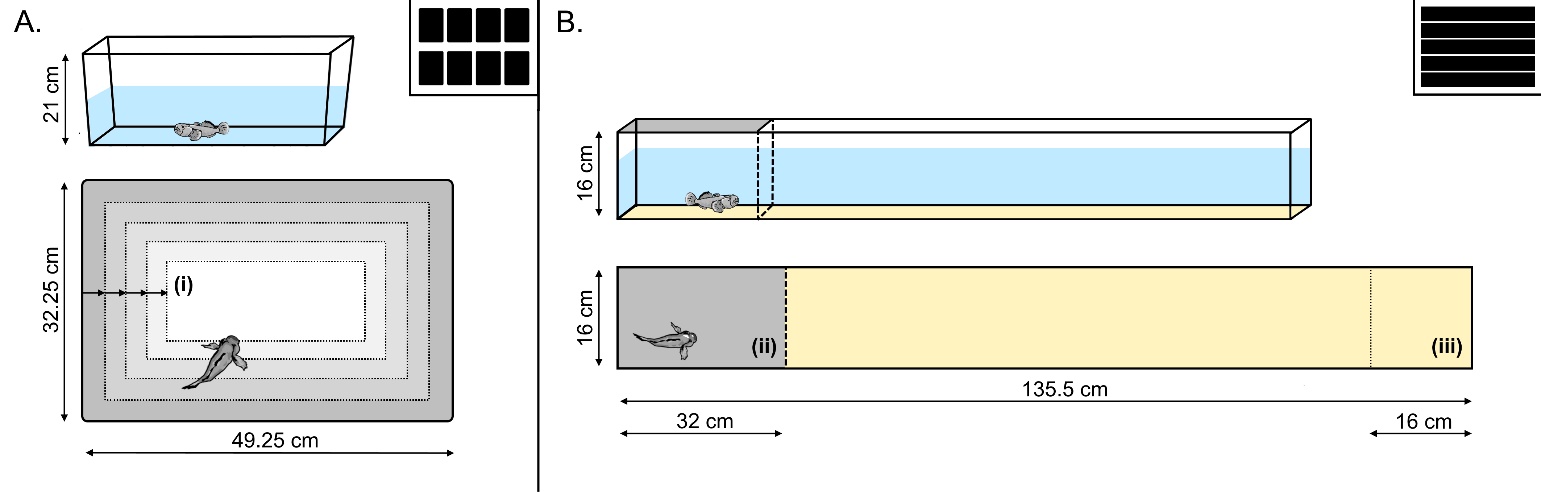
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| Table 1: Behaviour variable descriptions, distributions/transformations, and raw and (add for what) adjusted repeatability estimates. | | | | | |
| Assay | **Variable**  *(unit)* | **Description** | **Distribution** | **Repeatability** | |
| **Raw** | **Adjusted** |
| *Activity* | Distance  *(mm)* | Total distance that the fish moved over the full 20 min trial period. |  |  |  |
| Time in motion  *(proportional)* | Proportion of trial period of that the fish was actively moving around the arena. (Note, the minimum tracked speed for the fish to be considered moving was set to 5 mm/s.) |  |  |  |
| Average speed  *(mm/s)* | Average speed of the fish across the trial, excluding periods where the fish was not actively moving around the arena. |  |  |  |
| Edge use  *(mm)* | Score of the approximate average distance of each fish from the arena’s walls during the trial, using the time fish spent in each edge zone (see Fig 2A, i.e. 0 – 2.5, 2.5 – 5.0 cm, … etc.). |  |  |  |
| *Exploration* | Emergence latency  (s) | Time for the fish to emerge from a refuge after the barrier is removed (see Fig 2B). Maximum latency is set at 2700 s (45 mins). |  |  |  |
| Endpoint latency  (s) | Time for the fish to reach the end of the exploration arena from after the barrier is removed (see Fig 2B). Maximum latency is set at 2700 s (45 mins). |  |  |  |

**Figure Legends**

*Figure 1*. Sampling occurred in a 100 x 200 m area parallel to the shoreline within the Guldborgsund strait (pink). The site is in south-eastern Denmark, between the islands of Falster and Lolland, which was one of the first areas of the Danish coastline invaded by the round goby (ca. 2009). The invasion has since continued north-west, both via the Øresund towards the eastern Swedish coast, and via the Great Belt strait towards the Kattegat and western Denmark.

*Figure 2*. Experimental designs for the (A) *Activity*, and (B) *Exploration* assays, and (inset) the arrangement of each arena type when running multiple assays concurrently. The *Activity* arena is an open opaque white box filled to 10 cm. 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]. The time spend in each of these zones is then used to calculate edge use variables. The *Exploration* arena is an opaque black lane lined with a thin layer of sand at the bottom. The arena includes an enclosed refuge [i.e. **(ii)**] at one end, where the individual fish are held for a short acclimation period, 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 enclosed refuge. The fish is then taken to have reached the endpoint of the arena once >50 % of it’s body length has entered the final 16 x 16 cm ‘end-zone’ section of the arena [i.e. **(iii)**].

*Figure 1*

*Figure 2*