**Quantifying among-individual behavioural and trophic variation in the invasive round goby**

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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 how they interact with their environment and determine the outcome of trophic/food web interactions, including what they eat, how vulnerable they are to being eaten and who they compete with. Quantifying individual variation in behavioural traits is often a time-hungry and resource-demanding process, requiring robust sample sizes, repeated trials and individual identification. Coupling this approach with methods to measure individual variation in their trophic state in the wild (e.g. stable isotope analysis) is particularly challenging. Such processes require tissue removal from individuals and have the potential to induce behavioural changes in individuals. Nonetheless, there is a clear need to quantify behavioural-trait effects on ecological interactions. For the invasive species such as the round goby (*Neogobius melanostomus*), populations in invaded marine-brackish 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 individual approach is needed to quantify their impacts on the communities of recipient ecosystems. This study presents a novel methodological approach, combining laboratory-based behavioural tests with field-based stable isotope analysis of food web interactions, focusing on an established invasive population of round gobies in the Southwest Baltic Sea. We find that substantial individual variation in trophic state and behavioural state in this species, ….., This highlights that individual round gobies differ may significantly in how they are impacting invaded communities, and that behavioural variation may be a key component of this. Furthermore, this demonstrates that experimentally quantifying behavioural-trophic correlations is a viable approach for exploring individual-level ecological variation in wild populations.

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

**Introduction**

Intraspecific behavioural variation is closely linked to many ecological processes, including diet, predator-prey dynamics, 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 consistent behavioural variation (i.e. animal personality) is commonly expressed across a wide range of taxa (Gosling, 2008). Among-individual variation in risk-taking behaviour is often observed, where the terms ‘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 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). As such, variation in risk-taking can be associated with differences in feeding behaviour and vulnerability to predation (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.

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 particular challenge to analysing individual level trophic state and behavioural variation, is the need to remove animals from their environment and conduct minor, although invasive procedures (e.g., gut content analysis, isotope analysis)….

(carbon-12 – carbon-13; hereafter δ13C)

The role of individual variation in ecological interactions is particularly pertinent to the round goby (

There is a lack of studies that quantify how behaviour trait variation (i.e. risk-taking behaviour) translates to functional ecological variation in the wild. Therefore, the primary goal of this study is to test an approach to experimentally quantify both trophic state variation in animals in the wild, and individual behavioural variation in the laboratory in the same individuals. Specific we sought to quantify among individual variation in bold-exploratory behavioural traits and the trophic state in an established wild goby population. We predicted that round gobies would show among-individual variation in behavioural variables in bold-exploratory assays (activity, edge use, and emergence-exploration latencies), as well as among-individual variation in trophic state (i.e. in δ13C and δ15N values). We also conducted two additional exploratory analysis to test how individual behavioural variation influenced round goby interactions with prey species. First, we calculated correlations-covariation between individual isotopic values and round personality traits (i.e. those that show among-individual variation) to identify traits that were related to their trophic interaction. Second, we use stable isotopic analysis of round goby prey species and mixing models to quantify the relative contributions of prey taxa to round goby diets, and explore how individual behavioural variation may affect the round gobies impacts on prey.

**Methods**

1. *Behavioural-trophic variation experiment (Guldborgsund)*
   1. *Field Sampling*

To quantify bold-exploratory behavioural and trophic variation in a well-established invasive population, both round gobies and their potential prey community were sampled over 16-17 June 2020 from a shallow brackish estuary (Guldborgsund, 54.69645°, 11.84067°, Fig 1). Guldborgsund is one of the first Danish marine areas invaded by the round goby, first being observed ~2009, so can be considered a well-established population characterised by high population density where gobies are likely to experience strong intraspecific competition (Azour et al., 2015).

Round gobies were collected from a coastal estuarine habitat within the shallow brackish estuary, over a 2 ha (100 x 200 m, depth < 2.0 m, sandy with scattered-boulder substrate). Round gobies occupy shallow rock habitats over the Spring-Summer breeding period (Marentette et al., 2011), and are particularly active in April – June (Brauer et al., 2020). Gobies may show high site affinity, particularly around rocky structures (Lynch and Mensinger, 2012; Christoffersen et al., 2019). As tissue isotopes reflect assimilation of prey isotope ratios over a period of weeks to months (Thomas and Crowther, 2015), it is assumed that isotopic variation reflects their diet within Guldborgsund.

Fish were collected using a combination of fyke nets (… m, … mm mesh size), and baited traps (box 44 x … x … cm, … mesh size, cylinder 60 x 30 cm, … mesh size), to minimise personality biased sampling (Biro and Dingemanse, 2009; Michelangeli et al., 2016). Eight replicate sets of nets were deployed for 24 hours, even spaced across the sampling area (Fig. 1). Active sampling via push nets (width 65cm, mesh size 10 mm) was also attempted but was unsuccessful, although passive sampling alone has previously performed well at capturing unbiased samples in round gobies (Thorlacius et al., 2015). Fish > 80 mm total length (TL) were targeted for individual behavioural/ trophic analysis, as round gobies above this size have developed the adult morphological features required for feeding appropriate on hardbodied prey (i.e. gastropods, bivalves, (Andraso et al., 2011)

Fish were transported to DTU Aqua fish stable facility (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 (???). Laboratory salinity is within the natural range of the source location (Feistel et al., 2010), and is well within the osmoregulatory tolerance of the species [(Behrens et al., 2017; Puntila-Dodd et al., 2021)](https://www.zotero.org/google-docs/?PMzkql)

Prey fauna were sampled 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 … cm); shrimp net pushes (net width 65 cm, mesh size 1cm); 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 samples included benthic infauna, sessile fauna and mobile fauna. Samples were rinsed through a 0.5 mm sieve and rinsed with deionised water. One of each sample type was collected at each of the eight replicate sampling points within the sampling area (Fig. 1).

Samples of primary producers were also collected provide additional context to any observed isotopic variation. This includes three replicate samples were collected by hand of dominant algae types. Coarse organic matter water taken from core and quadrat samples, which was primarily from woody or leaf 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 or 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 (REF). All samples/packed filters were then frozen (-40 °C) before further processing and analysis.

* 1. *Tagging and fin clips*

Tissue samples were taken from round gobies, and individuals were tagged two days after returning to the laboratory. 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.) following standard procedures (Jørgensen et al., 2017). Round gobies cope well with internal tagging, showing no effects on survival and growth due to PIT tagging (Ruetz et al., 2006). Caudal fin tissue samples were taken concurrently with tagging, as a non-lethal method for obtaining individually identifiable isotopic samples (Hayden et al., 2015; Britton and Busst, 2018). The extreme outer edge of fins were taken using surgical scissors, and separated into three replicate samples per individual and stored individually prior to analysis. Although either procedure is not expected to influence of behavioural trait measurements, the combination of procedures may cause additional stress, therefore we conducted additional experiment to test the effect of combined tissue sampling and fin clips (see supplementary materials S1). No significant behavioural effects were found 10 days post-trial.

* 1. *Behavioural analysis*

Treatment groups were held in 3 tanks (enriched), with tank ID recorded as a potential covariate to account for subtle tank effects.

Sex, weighing

Two types of behavioural experiments were used to characterise individual behavioural variation, an *Activity* assay and an *Exploration* assay. To avoid effects of sociality, all assays were run with single individuals alone (REF). Similarly, water was entirely replaced between trials in the *Activity* to avoid carryover effects between trials due to odour signals, while as the *Exploration* assay was run in a continually flowing system in an input of uncontaminated water, which was flushed through with water between trials to minimise carryover odour effects between trials.

In the *Activity* trial, individuals were placed by hand into a 32.5 x 50 cm open field arena. Eight arenas were used in a 2 x 4 grid, to run multiple trials simultaneously. Following a 5 min acclimation period, individuals were filmed for 20 mins.

Fish movement behaviour was tracked using Toxtrac (v2.90, [(Rodriguez et al. 2018)](https://www.zotero.org/google-docs/?NPY2zs)). To account for potential among-arena sampling error due to parallax distortions, pixel/mm ratios for each arena were calculated for manual .

The following variables were extracted for further analysis: **average speed (mm/s), proportion of time spent moving (%), total distance travelled (mm) and edge use (time, s, spent > 10 cm from the outer edge of the arena,).**

The order of the assays.

* 1. *Stable isotope processing*

Samples were processed following standard SIA methodologies for marine aquatic food webs (Jardine et al., 2003). All field collected samples were placed on ice for transport (approx. 2 hrs). Field samples and fin clips held at -40° C in the laboratory. Invertebrates were identified to family level where possible, based on historical records of the invertebrate community in the region.

Invertebrates

* Grouped to relatively course taxonomic groups
* 3 replicates per group
* *Behavioural assays*

Fin clips, tail fin taken from the outer 5mm of the fin, generally showed no sign of infection.

* Any sections of the fins with visible parasite infections were removed under a dissecting microscope. Help in 2ml eppendorfs.
* Rinsed thoroughly with deionised water to remove any surface contamination.
* Drying: 60 degrees, 48 hours
* Sections composed of both fin ray and soft tissue, so sections ground into homogenous in tube using steel rods.
  1. *Statistical analysis*

**Results**

**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).

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**Tables**

**Figure Legends**

Figure 1. Main map, includes collection sites Guldborgsund (A) and Karrebaek (C), in relation to the Baltic Sea and the housing laboratory at DTU Lyngby (C). Inset top right, includes specific locations of collection sites A and B within their estuaries. Inset middle right, the 200 x 100m (2 ha) sampling area within Guldborgsund (black horizontal zone), with specific locations of eight sampling replicates (black triangles) within the area.

Figure 1

