**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**

Behavioural differences among individuals of the same species are an important source of variation that influences how an animal and their population interacts with their environment. Quantifying individual variation in behavioural traits is often a time- and resource-consuming 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, such as stable isotope analysis, is challenging due to the need to remove tissue from individuals, and the potential for procedures to induce behavioural changes in individuals. Nonetheless, there is a clear need to quantify behavioural-trait effects on ecological interactions. For example individual behavioural variation (e.g. bold-exploratory traits) is linked to invasion processes, and is linked to trophic/food web interactions, therefore an individual approach is needed to properly understand the trophic impacts of a marine invasive species such as the round goby (*Neogobius melanostomus*). This study presents a novel methodological approach, combining field-based stable isotope analysis of food web interactions with laboratory-based personality trait analysis in 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, ….. and that experimentally quantifying both is a viable approach to exploring the impacts of individual trait variation in ecosystems.

**Keywords**

**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 trial 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 aims of our analysis are as follows:

(1) 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 show consistent among individual variation in behavioural variables in bold-exploratory assays (activity, edge use, and emergence-exploration latencies), as well as among-individual carbon and nitrogen isotope variation.

(2) To test effects of individual tagging and tissue sampling procedures on round goby behavioural traits. We predicted that PIT tag and fin clip procedures have no effect on activity and edge use behavioural traits over short- (2 day) and medium-time periods (10 day) post-procedure. Additionally, we tested for growth and survival effects over a longer (10 week) period and predicted no treatment effect.

In addition to the specific hypotheses above, we also sought to validate the use of fin clips to estimate the recent diet of round gobies, and estimate δ13C and δ15N isotopic discrimination factors for fin and muscle tissue of round gobies. These values were used to conduct an exploratory analysis to measure the influence of behavioural trait on round goby’s diets, by testing for correlations/covariation between personality traits and trophic/diet variation.

**Methods**

Two populations were used for these experiments. 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°). These fish were also maintained in the laboratory, to measure their isotopic discrimination factor of fin and muscle tissue in relation to a standardised laboratory diet. Concurrently, to test for effects of effects of tagging and fin clips on round goby behaviour in the laboratory, round gobies were collected from a local fisherman on 1 October 2020 from Karrebæk Fjord (55.1923°, 11.67241°).

Guldborgsund is one of the first Danish marine areas invaded by the round goby, first being observed ~2009, while Karrebæk was invaded soon after (~2011), so both can be considered well-established populations characterised by high population density and strong intraspecific competition (Azour et al., 2015).

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

Round gobies were collected in 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; although see 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 … x … x … cm, … mesh size, cylinder … x … x … 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 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. This included:

* 1. *Tagging and finclip treatments*

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Transport and Holding conditions.

Treatments were applied immediately after fish undertook their first behavioural assay (*day 0*), following an extended period of acclimation (40 days, 1/10/20 – 10/11/20) to minimise any confounding effects of laboratory acclimation on behavioural responses and survival. Fish (n = 48) were subject to one of three tagging/sampling treatments: Control (no-PIT tag, not tissue sampling); Tagged (PIT tagged); Fin-clipped (PIT tagged and tissue sampled). Due to the low number of fish available, a non-factorial treatments design was used.

* 1. *Behavioural experiment*

Three behavioural trials were conducted to measure short and medium-term behavioural effects of tagging and sampling; one pre-treatment (*day 0*) and two post-treatment (*day 2* and *day 10*). Previous studies have shown little to no physiological effects over …….. A common open field/ novel environment assay was used to measure activity-exploratory behavioural variables.

* 1. *Survival and growth response*
  2. *Data analysis*

Continuous moderators were z-transformed to aid interpretation (Schielzeth, 2010).

1. *Guldborgsund Experiment* 
   1. *Fish and community sampling*
2. *Behavioural experiments*

Prior two behavioural experiment, all fish were tagged

1. *Feeding experiments*
2. *Stable isotope analysis*

Invertebrates were identified to family level where possible, based on historical records of the invertebrate community in the region.

1. *Data analysis*

Sex, weighing

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

1. *Isotope processing*

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.

Invertebrates

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

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.

**Results**

**Discussion**

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**Authorship**

**Data** **Accessibility**

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

