# How to Quantify Behavioural and Trophic Variation Among-Individuals: A case study of the invasive round goby

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## Abstract

An 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 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.

Few studies have directly measured how behavioural trait variation (i.e. activity, bold-exploratory traits etc.) translates to functional 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 quantify among-individual variation in bold-exploratory behavioural traits and the trophic state in an well-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 exploratory analysis to test how individual behavioural variation influenced round goby 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, and explore how individual behavioural variation may affect the round gobies impacts on prey.

## Methods

### Field Sampling

Round gobies and their potential prey community were sampled from 16-17th June 2020 from a shallow brackish estuary (Guldborgsund, 54.69645°, 11.84067°). Guldborgsund is one of the first Danish marine areas invaded by the round goby, first being observed ~2009. So this is may be considered a well-established self-sustaining invasive population, which is characterised by high population densities where round gobies are likely to experience strong intraspecific competition (Azour et al., 2015). Sampling took place 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 from April to June (Brauer et al., 2020). Gobies can show high site affinity, particularly around rocky structures (Lynch and Mensinger, 2012; Christoffersen et al., 2019), therefore it is assumed that isotopic variation of individuals collected in June will be primarily linked to their local diet within the estuary.

Round gobies were collected using a combination of fyke nets (4x large double fykes: 3 m funnels, 0.5 cm mesh size, 8 x 0.6 m wing, 2 cm mesh; 4x small double fykes: 2 m funnel, 1 cm mesh, 5 x 0.4 m wing, 2 cm mesh), and baited traps (8x box traps: 44 x 25 x 25 cm, 0.5 cm mesh size, 6 cm aperture; 8x cylinder traps: 55 x 30 cm, 0.5 cm mesh size, 10 cm aperture). Baits consisted of commercial frozen seafood mix of clams, mussels and shrimp (*Mytilus* sp., *Veneridae* spp. *Pandalus* spp.), mixed with canned sweetcorn and packed in fine knit elastic stocking material, so that fish could not consume the bait itself. Eight sets of nets were deployed for 24 hours, evenly spaced across the sampling area (for further details see supplementary materials S1). Multiple capture methods were used to minimise personality biased sampling (Biro and Dingemanse, 2009; Michelangeli et al., 2016). Active sampling via push nets and large dip nets was also attempted but was unsuccessful, although passive sampling alone has previously performed well at capturing unbiased samples in round gobies (e.g. (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 items (i.e. gastropods and bivalves (Andraso et al., 2011), so that any observed trophic variation is not linked to this ontogenetic transition.

Fish (n = 55) 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 (3mm Ivory Ex composite pellets, Aller Aqua, Denmark). Laboratory salinity 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 (REF). 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 also sexed, weighed, measured (total length, ‘TL’) just before tissue sampling and tagging, as well as being inspected for eye parasites and scored for their external parasite load (as percentage coverage of fins of fungal, nodules etc.), with the full process taking under one minute per fish.

### 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 infections/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.

### Behavioural analysis

Trials consisted of two types of behavioural assay (*Activity* and *Exploration*), each repeated three times at one week intervals. Before and during behavioural trials, fish were split approximately evenly between three round 800L (… x … 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 impacts 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 1A) (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). The eight arenas are arranged in a 2 x 4 grid under a standard laboratory fluorescent light in an area separated by a curtain, so arenas has consistent light conditions (~ 45 lux) and external sound/light effects were negligible. Behaviour is filmed using a modified web camera (Logitech BRIO 4K Ultra HD webcam, Logitech, Switzerland), positioned centrally 2 m above the arenas. Behaviour is tracked for 20 minutes, 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 odour cues 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 minimize among-arena variation due to parallax distortions, pixel/mm ratios for each arena were calculated using ImageJ v1.52a (Schneider et al., 2012) to manually calibrate each arena for tracking. Variables extracted were those related to their activity level and edge use in the arena (see detail in Table 1), where more activity in a novel environment and greater use of more exposed central areas is generally considered higher-risk or exploratory/bold behaviours (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 1B) (Toms et al., 2010). Individual fish were introduced into an (… x … cm) enclosed refuge at one end of a narrow arena (… x … cm, water depth = …). 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 able to be removed simultaneously from a position behind the refuges, so that fish were blind to the observer’s movements. Behaviour was then filmed as above for 45 minutes, with the camera positioned centrally 1.5 m above the arenas. *Exploration* arenas in this assay are connected to an inflow (approximately … L/min from the refuge end) so that filtered experimental water is continuously introduced through the trial period, and arenas are also complete flushed through with filtered water between trials to avoid carryover effects between trial rounds.

Behaviour in the *Exploration* assay was manually scored from videos, such as the latency to emerge and reach the endpoint of the arena (Table 1). Where fish are faster to enter 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 were weighed and had their PIT tag number recorded to confirm their individual identity, and were inspected for any physical injuries or general lethargy that may impact their behaviour in the 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 effects between trials (Bell, 2013).

### Statistical analysis

Behavioural repeatability

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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Sarah Wexler

## 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** | **Description** | **Distribution** | **Repeatability** | |
| **Raw** | **Adjusted** |
| *Activity* |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
| *Exploration* |  |  |  |  |  |
|  |  |  |  |  |  |

**Figure Legends**

Figure 1. Experimental designs for the (A) Activity, and (B) exploration assays.

**Figure 1**