Trap versus net: Behavioural sampling bias caused by capture method in three-spined sticklebacks

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ABSTRACT

Wild-caught animals are often used in behavioural or other biological studies. However, different capture methods may target individuals that differ in behaviour, life history and morphology, thereby giving rise to sampling biases. Here, we investigated whether juvenile three-spined sticklebacks caught in a natural population by passive and active sampling methods using frequently used tools (i.e. trap and hand net) differ in behaviours related to cognition and personality. The fish caught by traps were more prone to take risks and shoal (i.e. bolder and more sociable), but smaller in size and mass than the fish caught by hand nets. Individual variation in boldness was greater in the fish caught by hand nets, suggesting that this active sampling method may capture more representative samples of the natural population. Our results show the importance of capture method to avoid sampling bias in behavioural studies using wild-caught animals.

1. Introduction

Different individuals can exhibit consistently different behaviours across time and contexts within a population (i.e. behavioural types; Sih et al., 2004; Réale et al., 2007). For example, some individuals are more active, more aggressive, and bolder than others in various contexts, such as feeding, antipredator, and social behaviours (Sih et al., 2012; Wolf and Weissing, 2012). In empirical studies of animal behaviour, it is important to sample and use a representative subset of individuals in the population. However, most sampling methods potentially select non-random individuals (Biro, 2013). Personality may be one of the most important drivers of sampling bias, and different capture methods may select individuals with different behaviour types according to their probability of capture (Biro and Dingemanse, 2008). Evidences for this behaviour-related sampling bias has been found across taxa, including fishes (Cooke et al., 2007; Wilson et al., 2011), birds (Garamszegi et al., 2009; Camacho et al., 2017) and mammals (Réale et al., 2000; Boon et al., 2006). Nevertheless, few studies have compared behavioural sampling bias between passive and active capture methods.

Most studies of capture bias have focused primarily on passive sampling methods (e.g. minnow traps and nest-box traps) that require active behavioural involvement of target animals for successful capture (Carter et al., 2012; Stuber et al., 2013; Burns and Bonier, 2020; Kressler et al., 2021). For example, bold and active individuals that exhibit high levels of exploration and risk-taking behaviours are more likely to move into or toward traps (Biro and Dingemanse, 2008). On the other hand, active sampling methods chase or tow (e.g. sweep nets and seines) in pursuit of the target species, and so potentially capture a wider range of individuals than passive sampling because the target animals’ behavioural involvement in this process is none or minimal (Michelangeli et al., 2016). Nevertheless, active methods may also result in sampling bias if, for example, more active or faster-moving individuals escape more easily (He, 1993; Winger et al., 1999).

Here, we evaluated behavioural sampling bias of an active and a passive method in the three-spined stickleback (Gasterosteus aculeatus), an important and popular model species for behavioural, ecological and evolutionary studies (Bell and Foster, 1994; Ostlund-Nilsson et al., 2006; Wootton, 2012). For this, we captured juvenile sticklebacks from a natural population using two different and commonly used tools, traps (a passive method) and hand nets (an active method), and compared their behaviours, including cognitive ability, exploration, boldness and sociability, as well as their body size and mass. A recent study using captive sticklebacks showed that passive traps are associated with sampling bias (Kressler et al., 2021). We expected that behaviours of fish entering into the traps would be biased toward high levels of boldness, exploration and cognitive ability, whereas active sampling using hands nets would capture fish with higher variance in their behaviour patterns in the population.

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2. Material and methods

2.1. Capture methods and rearing conditions

We captured three-spined sticklebacks using traps and hand nets in the Río Sar (Galicia, Spain) on 14th October 2020. This is an annual population, and at this time of year, there are juvenile sticklebacks only (Kim et al., 2017). Juvenile sticklebacks tend to form shoals for protection against predators (Pitcher, 1986). We carried out trap and net samplings for two hours at the same time in the morning within a 250 m distance. Two people went into the river and captured 69 fish by active sampling, each working independently with a 35 cm × 30 cm hand net (5 mm mesh size; brown colour; 1.5 m length of handle aprox.). Since fish were not visible due to thick vegetation in the river, in each sampling attempt we netted a random spot by sweeping the riverbed for about 10 s then collected any caught fish from the net within 15 s. For trap sampling, we used three funnel-shaped traps (see Supplementary Fig. S1) baited with a mixture of bloodworms and blue cheese (Meril, 2015). The traps were placed at randomly selected spots on the riverbed (see Supplementary methods and Fig. S2). After about 10 s then collected any caught fish from the net within 15 s. For trap sampling, we used three funnel-shaped traps (see Supplementary Fig. S1) baited with a mixture of bloodworms and blue cheese (Meril, 2015). The traps were placed at randomly selected spots on the riverbed (see Supplementary methods and Fig. S2). We captured 21 fish by this passive sampling method. The fish captured by the two methods were transported in two separate 25-L containers to our laboratory facility. Fish were acclimated to laboratory conditions and housed in 8-L individual tanks under a constant photoperiod of 11 h:13 h light:dark cycle and temperature of 15 °C, which simulated seasonal conditions in their natural habitat. Photoperiod and temperature were controlled by a programmed LED illumination and flow-through water cooling system.

2.2. Behavioural assays

2.2.1. Cognitive ability assay

Nine days after sampling, the cognitive ability of fish that survived the acclimatization period (trapped fish: N = 19; netted fish: N = 68) was evaluated by a detour-reaching task in which the fish needed to find the entrance in an apparatus to reach the food (Minter et al., 2017; Álvarez-Quintero et al., 2021) (see Supplementary methods and Fig. S2). During the test, which lasted a maximum of 180 min, we registered whether the fish entered the cup as well as the time taken to enter. We assigned the maximum time (180 min) to the fish that did not enter the cup (trapped fish: 3 out of 19; netted fish: 16 out of 68).

2.2.2. Exploration, boldness and sociability assays

Each fish was subjected to three consecutive behavioural assays on the same day (exploration, boldness, then sociability) 12–18 days after capture (mean = 15 days). One trapped fish died before the behavioural assays. All behaviours were assessed in the same observation tank, which was partitioned into different inner compartments by removable barriers and adjusted for each test (illustrated in Supplementary Fig. S3). The observation tank was illuminated with LED light. Details of the behavioural assays are described in Supplementary Fig. S3. Briefly, the focal fish was netted from its holding tank and placed inside an opaque cylinder located in the observation tank. After acclimatization of 30 s, the cylinder was removed, and the focal fish could swim freely throughout the tank (hereafter the “arena”) for 180 s. We quantified the total time and distance moving, the proportion of the arena explored, and the total time spent by the focal fish close to the walls (< 3 cm) to determine its exploratory behaviour (Bell and Stamps, 2004). Then the fish was enclosed inside a transparent cylinder, and the tank was prepared to assess its boldness. The observation tank for the boldness assay contained an acclimatization zone surrounded by a refuge area with artificial plants on one side of the compartment. After acclimatization of 30 s, the fish was allowed to exit the refuge area and freely swim in the arena. We measured the time taken for the fish to leave the refuge for up to 10 min and the total time spent in the arena (outside the refuge) during the following 3 min to determine its boldness (Jolles et al., 2016). The fish that did not exit the refuge within 10 min were assigned a latency-to-exit score of 10 min and time spent in the arena of 0. Then the fish was positioned again using a transparent cylinder for the sociability assay. The tank for the sociability assay consisted of two compartments, a focal fish zone, and a stimulus fish zone, which contained four juvenile sticklebacks. The focal fish was first positioned in the focal fish zone using a transparent cylinder then released following acclimatization of 30 s. We measured the total time spent in the socialization area close to conspecifics for 3 min (Jolles et al., 2015). Each focal fish was used as a stimulus for four consecutive sociability assays of other individuals after first itself being tested for sociability. There was no consistent difference in size (i.e. standard length) between the focal fish and four stimulus fish (paired t-test: t104.36 = 0.025, p = 0.98; mean length ± SE: 32.5 ± 0.33 mm, N = 68). Once the behaviour assays finished, each focal fish was netted then weighed to the nearest 0.001 g using a digital balance (GRAM FH-100) and measured to the nearest 1 mm (standard length) using an ictiometer before returned to its home tank.

The behaviour assays were video-recorded at 25 fps and animal tracking was performed by using a homemade software coded in Python (v m3.7) using the opencv library (v4.5). The coordinates obtained were then analysed with R (R Core Team, 2018, v.3.6.2) to extract the study variables.

2.2.3. Statistical analyses

We compared variances of each trait among individuals between the two capture methods using Levene’s test of homogeneity of variances. We also compared the trait means between the capture methods using student’s t-tests. We compared the time taken to enter into the apparatus in the detour-reaching task and the latency to exit the refuge in the boldness assay of the two capture methods using Cox Proportional Hazard Models (CPHMs) implemented in R package survival (function coxph; Therneau and Grambsch, 2000), including the capture method as fixed factor. Censoring was used to identify whether a fish died or did not exit the refuge during the boldness assay or enter into the apparatus in the cognitive ability assay. Before the analysis, the proportion of time spent close to the conspecifics was arcsine-transformed to improve data distribution and meet model assumptions of normality.

3. Results

The proportion of fish that succeed to enter the apparatus during the cognitive ability assay was similar between the trapped and netted fish. We also compared the time taken to enter into the apparatus in the detour-reaching task and the latency to exit the refuge in the boldness assay of the two capture methods using Cox Proportional Hazard Models (CPHMs) implemented in R package survival (function coxph; Therneau and Grambsch, 2000), including the capture method as fixed factor. Censoring was used to identify whether a fish died or did not exit the refuge during the boldness assay or enter into the apparatus in the cognitive ability assay. Before the analysis, the proportion of time spent close to the conspecifics was arcsine-transformed to improve data distribution and meet model assumptions of normality.

### Table 1

| Trait                        | Levene’s F, p-value** | T-test’s T/√t, p-value** |
|-----------------------------|-----------------------|--------------------------|
| Detour-reaching task        |                       |                          |
| Exploration                 | 1.32, 0.25            | 1.26, 0.26               |
| Proportion of time moving   | 0.24, 0.63            | -0.61, 0.55              |
| Total distance swim         | 0.005, 0.94           | -0.41, 0.68              |
| Proportion of the arena     | 0.02, 0.89            | -0.79, 0.43              |
| Proportion of time spent    | 1.70, 0.19            | 1.49, 0.15               |
| close to the walls (< 3 cm) |                       |                          |
| Boldness                    |                       |                          |
| Latency to exit refuge      | 4.88, 0.03            | 5.30, 0.02               |
| Proportion of time spent    | 3.20, 0.08            | -1.02, 0.31              |
| in the arena                |                       |                          |
| Sociability                 | 3.79, 0.05            | -2.59, 0.01              |
| Proportion of time spent    |                       |                          |
| close to the conspecifics   |                       |                          |
| Body size (mm)              | 2.92, 0.09            | 2.59, 0.02               |
| Body mass (g)               | 0.16, 0.69            | 2.62, 0.04               |

* F statistics derived from Levene’s tests comparing variances of the mean between the two capture methods.

** T or χ² derived from t-tests and cox proportional hazard models (CPHMs). The significance of terms of the CPHMs was determined by the Likelihood Ratio Test (LRT).
especially in terms of their behaviour, which better represent the population variability.

As expected (Réale et al., 2007), trap-sampling targeted bolder fish than hand net-sampling. Previous studies have also found that the success of passive methods using either unbaited or baited traps rely strongly on individual personality in birds (Garamszegi et al., 2009), mammals (Réale et al., 2000; Boyer et al., 2010) and fishes (Wilson et al., 1993; Biro and Post, 2008). The exploration and inspection behaviours of target animals may be crucial for trapping. Bolder individuals may be more prone to explore (Réale et al., 2007; Garamszegi et al., 2009; Stuber et al., 2013) and consequently are more likely to be captured by traps, leading to behavioural bias in captured samples. In this study, sticklebacks captured by traps were more sociable. Previous studies of bluegill fish also have found that sociable individuals were selectively captured by angling (Louison et al., 2018, 2019). In the three-spined stickleback, sociable individuals tend to form groups, whereas less sociable individuals live in solitary (Wolf and Krause, 2014; Joles et al., 2020). Since in social animals grouping enhances foraging efficiency of individuals (Krause and Ruxton, 2002), sociable fish are probably more likely to detect and enter baited traps together with the others from the same shoal. However, a recent study of three-spined sticklebacks has found the opposite pattern, with less sociable individuals being the first to enter into a trap containing conspecifics (Kressler et al., 2021). Further studies are required to assess whether the behavioural sampling bias found in our study occurs also with other types of fish traps (Reinhardt and Hrodey, 2019). Smaller body size of the trapped fish may reflect increased risk-taking and willingness to access food in smaller (and perhaps hungrier) individuals (Bisi et al., 2011). It is also possible that smaller and younger fish are more frequently trapped because they tend to shoal more than older fish (Krause and Ruxton, 2002). Further studies need to explore the sex difference in sampling bias because female and male sticklebacks differ in size and behaviour from the juvenile stage (Velando et al., 2017).

Our study describes, for the first time, differences in behaviour and size of sticklebacks captured in the field by two commonly used capture techniques and suggests that passive sampling can give rise to behavioural bias. Our results suggest that it is important to carefully choose among different sampling methods to capture a representative sample of
the population, especially in studies of animal behaviour (Webster and Rutz, 2020). Furthermore, individual differences in behaviour may lead to differences in animals’ involvement and performance in studies of behaviour or cognition in captivity (Morton et al., 2013; Webster and Rutz, 2020). We also highlight that it is necessary to discuss about the possible effects of sampling methods and bias on the results in behavioural studies, especially when animals were caught by a passive sampling method only.

Ethical Considerations

This experiment was approved by the Animal Experiment Ethics Committee of the Universidade de Vigo and the Xunta de Galicia (ES360570181401/19/FUN01/BIOI AN.08/SYK).

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CRediT authorship contribution statement

NAQ planned the study. NAQ and VC performed the experiment and collected the data. NAQ developed the statistical analyses. All authors contributed to write the manuscript.

Data Availability

All data needed to evaluate the conclusions in this paper and/or in the Supporting information. The raw data can also be found in the Figshare digital repository doi.org/10.6084/m9.figshare.14635986.

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Conflict of Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.beproc.2021.104504.

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