Research Article

Current temperatures limit the potential impact of a commonly traded predatory gastropod

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Abstract

The pet trade has facilitated the spread of invasive alien species (IAS) globally, with negative consequences for biodiversity. The prediction of impacts is a major goal for invasion ecologists, and is especially crucial in an industry often lacking knowledge about traded species. We focused on the predatory gastropod *Anentome helena*, a species originating in south-east Asia and traded around the world, but with taxonomic uncertainty. We first set out to determine where our study organism fell within the *A. "helena"* species complex, known to comprise at least four cryptic species, before assessing the effect of temperature on the number of prey, the pulmonate snail *Physella acuta*, killed per predator via functional response experiments at two temperatures. We used 22 °C as a recommended temperature for housing the species in captivity, and 18 °C as a representative summer lake temperature in temperate climates of Europe. We also assessed the role of predator group size (1×, 2×, 3×) on predation (total consumption and average *per capita* consumption) at the experimental temperatures with fixed densities of prey, as well as the effect of these temperatures on prey activity. Our organisms belonged to a cryptic species originating from Thailand (*Anentome* sp. A), matching the findings of aquarium trade samples in other continents. In the functional response experiments, we found maximum feeding rate to be significantly reduced at the lower temperature. A similar result ensued from group feeding, with total consumption significantly reduced and the reduction in average *per capita* consumption approaching significance at the lower temperature. There was no significant effect of group size on the average *per capita* consumption in the group trial, indicating neutral conspecific interactions. No significant effect of temperature on the activity of the prey species was found, suggesting decreased consumption was mainly driven by predator, rather than prey. These results suggest limited *A. helena* impacts in the short-term, but increasing temperatures with climate change may facilitate greater consequences from releases. We suggest future studies assess other potential predatory impacts and survival across relevant abiotic conditions, and encourage the use of similar methods to assess the impacts of other commonly traded species.

Key words: *Anentome helena*, ecological impact, functional response, invasive alien species, molecular identification, pet trade

Introduction

The unrelenting spread of invasive alien species (IAS) is one of the major drivers of global biodiversity loss (Seebens et al. 2017; IPBES 2019). An increasingly globalised world has led to new pathways facilitating this movement of species, and the pet trade is one important driver of this development (Lockwood et al. 2019). While many species within the pet trade spend their entire lives in confinement, the risk of escape or release from producers, importers, retailers and owners is ever-present. Indeed, the pet trade has facilitated the establishment of non-native populations globally, and is deemed responsible for 53% of invasive vertebrate species (Saul et al. 2017; Gippet and Bertelsmeier 2021) and a third of all aquatic IAS (Padilla and Williams 2004), with escapes and releases by aquarists a major contributor of invasive freshwater species in Europe (Nunes et al. 2015). The industry is also known to be poorly regulated (Raghavan et al. 2013), with species often only identified to their genus names, or worse, misidentified (Shivambu et al. 2020b).

Molluscs, primarily gastropods and bivalves, are popular within the pet trade globally (Ng et al. 2016b; Banha et al. 2019; Shivambu et al. 2020a), for both ornamental appeal and algae control (Patoka et al. 2017). Releases have led to a number of high-profile mollusc IAS emerging from the pet trade with negative ecological impacts. For example, the South American golden apple snail (*Pomacea canaliculata* Lamarck, 1822) has established non-native populations in the United States, Japan, China, Taiwan, Thailand and Indonesia (Shivambu et al. 2020a), and is believed to have reduced native freshwater snail populations, become an agricultural pest, and acted as a host for parasites (Halwart 1994; Rawlings et al. 2007). Aquarium releases have also seen *Melanoides tuberculata* (O. F. Müller, 1774), a species with a broad African and Asian native range, and the south-east Asian *Tarebia granifera* (Lamarck, 1816) establish extensive invasion histories (Rader et al. 2003; Raw et al. 2015; Da Silva et al. 2019; Makherana et al. 2022), posing threats to native biodiversity. While the majority of gastropods in the pet trade are thought to be herbivorous or detritivorous, the "assassin snail" *Anentome helena* (von dem Busch, 1847; formerly *Clea helena*) is readily available (Patoka et al. 2017; Shivambu et al. 2020a) and in possession of carnivorous habits. Uncertainty surrounds its taxonomic identity, with *A. helena* thought to comprise a cryptic complex of at least four species (*Anentome "helena"*: Strong et al. 2017), however a sample from the US pet trade was found to be conspecific with those already established in Singapore, a global ornamental pet trading hub, which may indicate ubiquity of this species in the trade (Ng et al. 2016a; Strong et al. 2017). With concern over further non-native establishment elsewhere (Bogan and Hanneman 2013), its potential to exert impacts in ecosystems beyond its native range is in need of study.

With *A. helena* found to be amongst the most readily available aquatic gastropods in Germany (Dickey et al. in prep), we aimed to solve the taxonomic uncertainty by phylogenetically analysing our study organisms, and then by assessing the role of temperature on its potential predatory impact. While availability has been shown to be a suitable proxy for propagule pressure (Duggan et al. 2006; Chucholl 2013), abiotic barriers to growth and survival must be overcome (Blackburn et al. 2011), and abiotic tolerances, especially to low temperatures, have proven crucial to the establishment success of numerous pet trade species (Veselý et al. 2015; Standfuss et al. 2016). Therefore, we set out to assess how temperature affects the feeding rates of *A. helena* on *Physella acuta* (Draparnaud, 1805) prey, a wide-ranging aquatic pulmonate snail present in Europe since the 1700s (Vinarski 2017). We used two experimental temperatures: 22 °C, a recommended lower limit for keeping the species in home aquaria (e.g. Aquariumtips.org 2020; Interaquaristik.de 2022; Tierlexikon.info 2022), and 18 °C, a temperature representative of summer water temperatures in temperate climates such as in Europe. We tested *per capita* feeding rates under these temperature treatments using functional response experiments (i.e. measures of how prey consumption varies with prey density), which have been used effectively as a means of assessing the impacts of invasive species over the past decade due to their ability to incorporate a wealth of context dependencies (Dick et al. 2014, 2017). However, one potential limitation of this measure of *per capita* consumption is that it typically does not account for the role of conspecific interactions inherent in group foraging, something potentially important in the context of pet species releases, when small numbers of individuals might be released together. There are three broad categories of conspecific interactions: neutral, antagonistic (prey risk reducing: Livernois et al. 2019), and synergistic (prey risk enhancing: Livernois et al. 2019), and using different experimental predator densities (i.e. fixed prey densities with single, double and triple predators), we assessed how temperature affects group feeding rates. We also assessed the role of temperature on the activity levels of *P. acuta* in order to elucidate whether predator or prey were the primary drivers of any differences in consumption at the two experimental temperatures. In summary, our study objectives were to 1) determine where our *A. helena* study organisms fell within the *Anentome "helena"* species complex; and test: 2) whether temperature would affect *per capita* consumption of *A. helena* using functional response experiments, 3) whether temperature and/or group size would affect feeding rates (total consumption and average *per capita* consumption) using our group feeding tests, and 4) whether our experimental temperatures would affect the activity levels of the prey species, *P. acuta*.

Methods

Anentome helena (Figure 1) were ordered online (garnelen-direkt.de) and arrived on 15th March 2022. The snails were held in an 18 °C lab with an 8-hour light regime for five days in a 56 L glass tank (i.e. 60 cm × 30 cm × 30 cm length, width and height) containing a 1 cm-deep layer of sand, and a heater (Eheim, Germany) and probe (Aqua-medic Titanium, Germany) to maintain the water temperature at 22 °C. *Anentome helena* were held under these conditions for five days before being split into two groups, i.e. a "warm" group and a "cold" group. On day six, the temperature of the "cold" group was dropped to 20 °C, and further to 18 °C on day seven. Temperature was adjusted via thermostat, so no additional disturbance of the snails in the "cold" group occurred. Snails in both groups were fed once every three days with frozen mysids (Petman Premium, Germany). The prey species, *P. acuta* (Figure 2), were ordered from Shrimpfarm-Frankfurt.de and arrived on 10th March. They were held in a covered plastic aquaria (approx. 50 *P. acuta* per 2 L, 18 cm × 12 cm × 12 cm) with an air stone and wooden sticks for habitat complexity, and fed "Veggie Wafers" food pellets (Tetra, U.S.A.) every three days. They were held in the same 18 °C lab as *A. helena*.

Molecular identification of *A. helena*

DNA was extracted from the foot tissue of ten snail specimens using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. A fragment of the mitochondrial cytochrome *c* oxidase subunit I (*COI*)

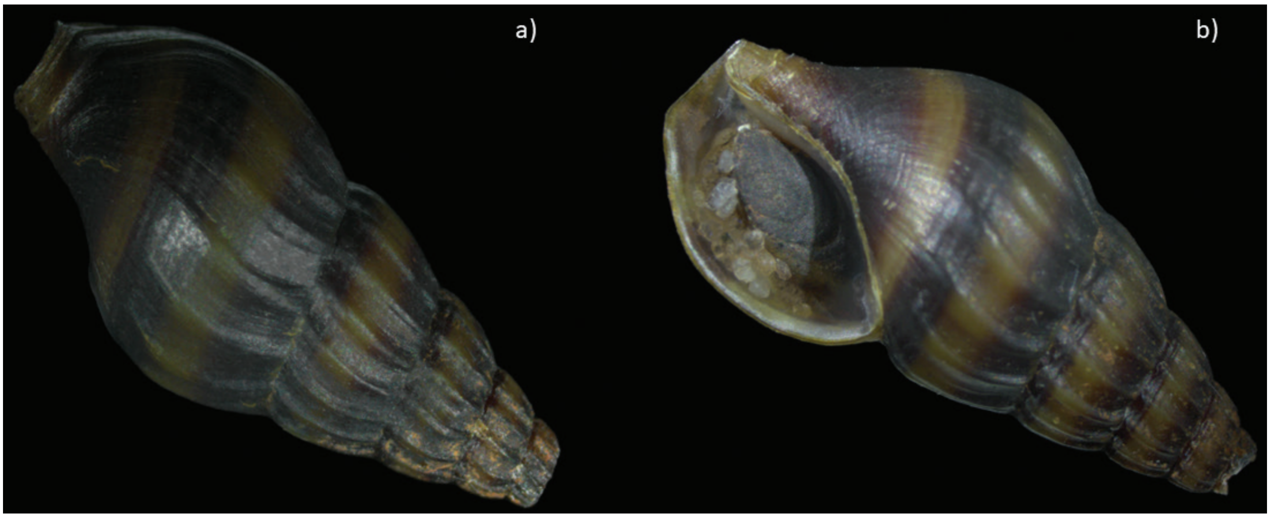

Figure 1. Photographs of predator *A. helena*. Morphological similarity noted between our study individuals and *Anentome* sp. A in the *A. "helena"* complex (Strong et al. 2017).

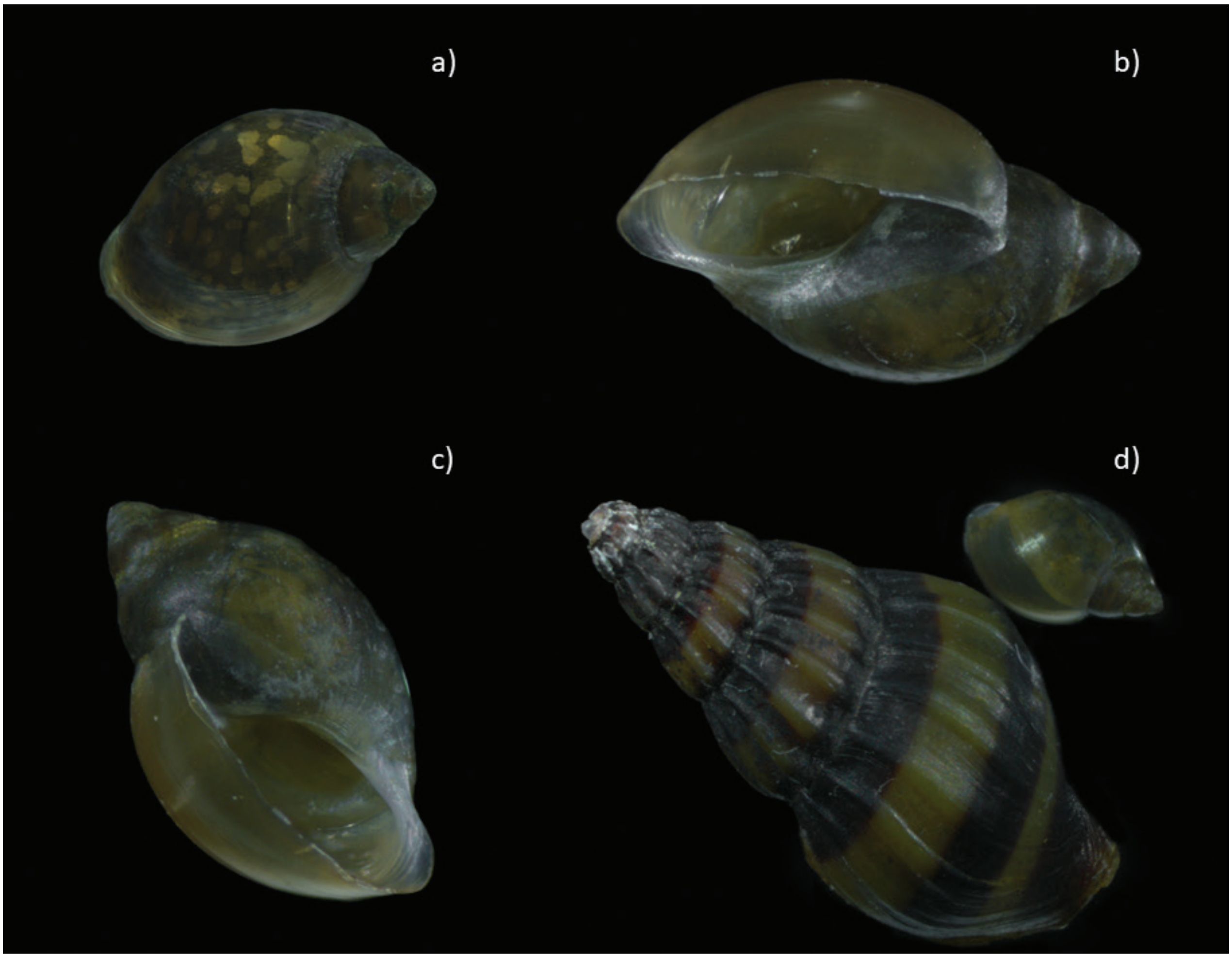

Figure 2. Photographs of prey *P. acuta* specimens (**a–d**) with a demonstration of relative size between the predator, left, and prey (**d**).

was amplified using primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). PCR reactions were conducted in 10 µL volume reactions, containing 1 µL of forward and reverse primers (5 mM concentration), template DNA, 10X PCR buffer (Invitrogen, USA) and dNTPs, 0.1 µL Taq DNA Polymerase (Invitrogen, USA) and 4.9 µL of nuclease-free water. Amplification was performed under the following conditions: 94 °C for 3 minutes; 35 cycles of 94 °C for 45 seconds, 48 °C for 45 seconds, and 72 °C for 60 seconds; 72 °C for 7 minutes. Consequently, we obtained quality PCR product for one specimen. PCR product was sequenced on Sanger sequencing platform (Applied Biosystems, USA) at Eurofins Genomics (Kiel, Germany).

Raw forward and reverse sequences were assembled in CodonCode Aligner (v 3.7.1; Codon Code Corporation). *Anentome "helena"* consists of multiple, undescribed, cryptic species (Strong et al. 2017). To validate the species identification, we conducted a phylogenetic analysis using known samples representing the four cryptic species as well as the closely related *Nassodonta dorri* (Wattebled, 1886), with *Naytia granulosa* (Lamarck, 1822) as an outgroup; these samples were downloaded from NCBI (Suppl. material 1: table S1). Samples were aligned using MUSCLE v3.8.1551 (Edgar 2004). A maximum likelihood tree was constructed using MEGA v 10.2.6 with 1000 bootstrap replicates.

Functional response trials

Prior to trials commencing, snails from both groups were removed from the holding tanks and starved for 48 hours in 1 L of water within 2 L plastic aquaria (18 cm × 12 cm × 12 cm) containing a 1 cm depth of sand, an air line and covered with a plastic sheet, and held within the respective "warm" and "cold" water baths (77 L plastic container: 70 cm × 55 cm × 20 cm) containing a heater, probe and two circulation pumps (Tunze, Germany) to ensure even distribution of temperature (see Suppl. material 1: figure S1 for experimental set up). *Physella acuta* prey were sorted and given a 24-hour adaptation to the experimental conditions, also in 2 L aquaria with an air line and covered, and held in the baths. Experimental arenas (1 L plastic jars: 9 cm × 9 cm × 14 cm) were filled with 400 ml of water and added to the water baths 24 hours before trials began in order to give sufficient time for them to reach the desired experimental temperatures. During this time, and during the experimental trials, air lines were in the water to remove any potential confounding factors arising from warmer water holding less oxygen. On the day of the trials, the experimental prey densities (1, 2, 4, 8 and 16; n = 4 per prey density per temperature treatment) were sorted and added to the arenas 30 minutes before *A. helena*. Trials commenced upon the addition of a single predator to the arena and loosely closing the arena lid. Control trials (n = 4 per prey density per temperature treatment) involved the same preparation only without the addition of a predator to quantify *P. acuta* mortality for any other reason. Trials ran for 48 hours, after which they were ended by the removal of each arena's *A. helena*. The number of live *P. acuta* was subsequently counted to determine predator consumption.

Group feeding trials

After the commencement of functional response experiments, *A. helena* were returned to holding tanks with conspecifics and left undisturbed for 7 days. 24 "warm" and 24 "cold" *A. helena* were then selected haphazardly for group feeding trials and starved for 48 hours in covered, aerated 2 L aquaria (density of 12 snails per tank). 480 *P. acuta* were selected from holding tanks 24 hours before trials commenced and split into a "warm" group and a "cold" group, also held in covered, aerated 2 L aquaria. On the day of the trials, *P. acuta* were grouped into densities of 20 before addition to the experimental arenas (1 L plastic jars with 400 ml water as in functional response trials), 30 minutes before the addition of *A. helena*. Trials commenced upon the addition of *A. helena*, which were added to experimental trials in groups of 1, 2 or 3 (n = 5 per density), and the closing of the arena lid. Again, trials ran for 48 hours and the water was oxygenated throughout the trials to prevent any confounds from temperature or from snail density.

Physella acuta activity

To assess the effect of the two trial temperatures on the *P. acuta* prey, we measured activity in 100 ml water in round plastic dishes (dimensions: diameter 8.4 cm, height 4.2 cm). Each dish had a cross drawn across on the base, and activity was recorded using a CX action camera (ACTIVEON Inc., U.S.A.). Videos were watched back, with the number of line-crosses over a 20-minute experimental period counting as an indicator of activity. The experimental period began ten seconds after the final *P. acuta* per batch of four was added to its dish. The sample size was n = 16 per temperature treatment.

Data analyses

All analyses took place in R version 4.1.2 (2021-11-01). Functional responses were modelled using the “frair” package (Pritchard et al. 2017), with the type of curve (i.e. linear Type I, hyperbolic Type II or sigmoidal Type III) derived through logistic regression considering the proportion of prey consumed as a function of initial prey density, with a significantly negative first-order term indicative of a Type II, and a significantly positive first-order term followed by a significantly negative second-order term indicative of a Type III. Functional responses of each species at each salinity were modelled using maximum likelihood estimation (MLE; Bolker et al. 2009) and the random predator equation (Rogers 1972), due to prey not being replaced as they were consumed:

$$N_e = N_0(1 - \exp(a(N_e h - T))) \tag{1}$$

where N_e is the number of prey consumed, N_0 is the initial density of prey, a is the attack rate, h is the handling time and T is the total time available (i.e. 48 hours). Models were fitted to the data using the Lambert W function owing to the recursive nature of the random predator equation (Bolker 2008). The initial a and h estimates were non-parametrically bootstrapped (n = 2000) to construct 95% confidence intervals around the functional response curve for each treatment. Significant differences between attack rates and handling times can be determined by visual inspection of confidence intervals (Figure 4), or using the delta or difference method of Juliano (2001) (Suppl. material 1: table S3; see Pritchard et al. 2017).

In the group feeding trials, the effect of temperature and predator density on 1) total consumption and 2) average *per capita* consumption (i.e. total consumption divided by the predator density) were assessed using generalised linear models. For 1) a Quasipoisson distribution was used due to overdispersion, and for 2) a Gaussian distribution. In each model, backward eliminations of non-significant terms and interactions resulted in the most parsimonious fits (Crawley 2014). Post-hoc tests with Tukey p value adjustment were performed using the emmeans package (Lenth et al. 2020) to assess differences in total consumption between combinations of temperature and group size.

For the test of *P. acuta* activity, the effect of temperature on activity (i.e. the number of line crosses) was assessed using a generalised linear model with Quasi-poisson distribution.

Results

Molecular identification of *A. helena*

Phylogenetic analysis showed that our specimen belonged to *Anentome* sp. A, as determined in Strong et al. (2017) (Figure 3). This species originates from Thailand and other samples purchased through the aquarium trade in the USA have belonged to this clade (Strong et al. 2017). This suggests that aquarium animals from the USA and Europe are from a common source. Analysis using a neighbour-joining approach, not shown, produced the same results.

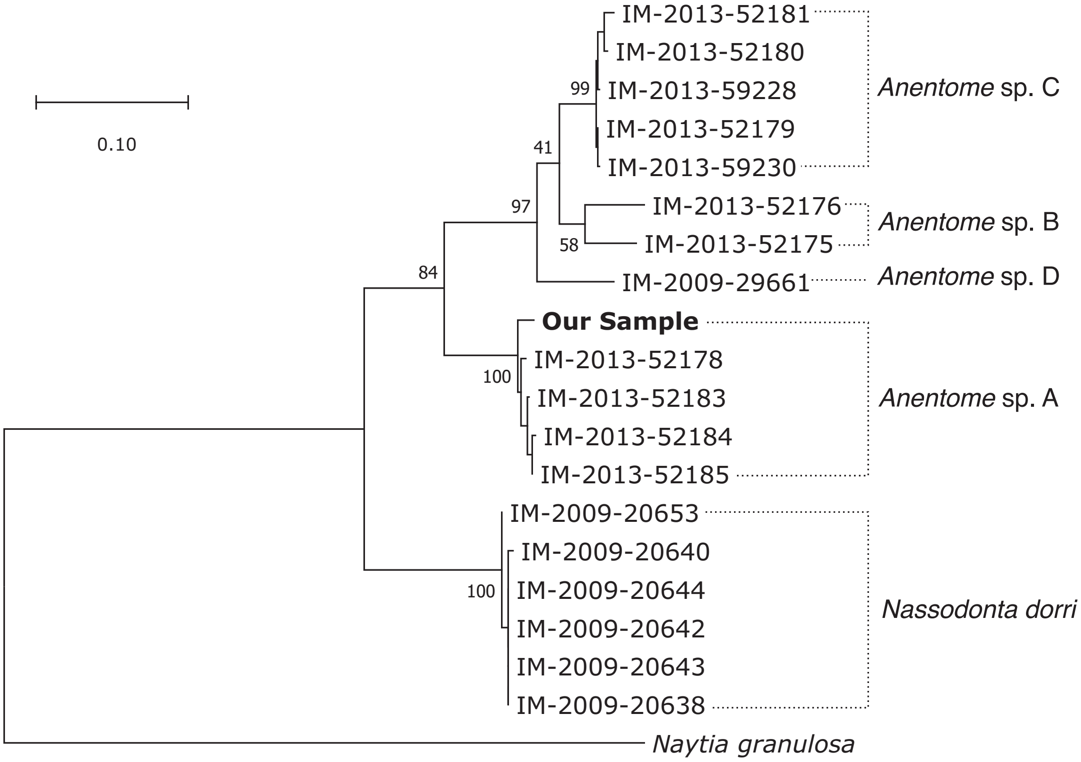

Figure 3. Maximum likelihood tree based on the mitochondrial gene COI. Bootstrap support values are shown at nodes and the scale bar represents the number of nucleotide substitutions per site. The sample used in this experiment, “Our Sample”, falls with *Anentome* sp. A. See Suppl. material 1: table S1 for sample information.

Functional response trials

Prey survival in all controls was 100%, and therefore all prey mortality was attributed to *A. helena* consumption. Type II functional responses were observed under both temperature treatments, as determined by significantly negative first-order terms (Suppl. material 1: table S2, Figure 4). There was a significantly greater value for the handling time parameter at 18 °C ($p < 0.05$; Suppl. material 1: table S3), and hence, a lower maximum feeding rate (i.e. $1/h$) than at 22 °C (22 °C: 4.264 consumed; 18 °C: 1.036 consumed).

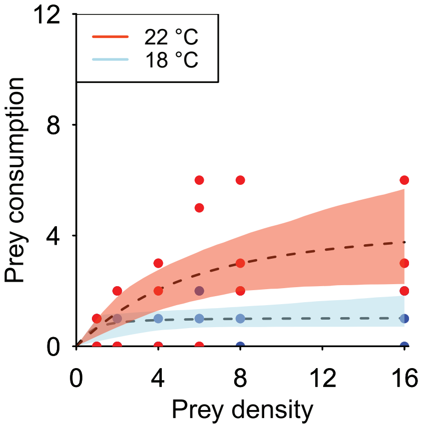

Figure 4. Functional Responses of *Anentome helena* towards *Physella acuta* prey at 22 °C and 18 °C temperature treatments. Experimental trials lasted 48 hours. Shaded areas are bootstrapped 95% confidence intervals.

Group feeding trials

There was a significant effect of temperature and group size on total consumption (adjusted $R^2 = 0.937$, $F_{3,26} = 144$, $p < 0.001$; Figure 5), but the two-way interaction was non-significant. Consumption at 18 °C was significantly less than at 22 °C (mean difference ± SE = -0.811 ± 0.321, $F_{1,26} = 6.551$, $p = 0.018$), consumption for groups of three was significantly greater than for single predators (mean difference ± SE = 1.308 ± 0.446, $t = 1.909$, $p < 0.01$), with the difference in consumption between groups of two and single predators approaching significance (0.896 ± 0.469, $t = 1.909$, $p = 0.067$). Post-hoc comparisons revealed significant differences in consumption between temperature-group size treatments (Suppl. material 1: table S4). When the effects of temperature and group size on *per capita* consumption were assessed, there was an effect approaching significance for temperature ($p = 0.054$), but no significant effect of group size ($p > 0.05$).

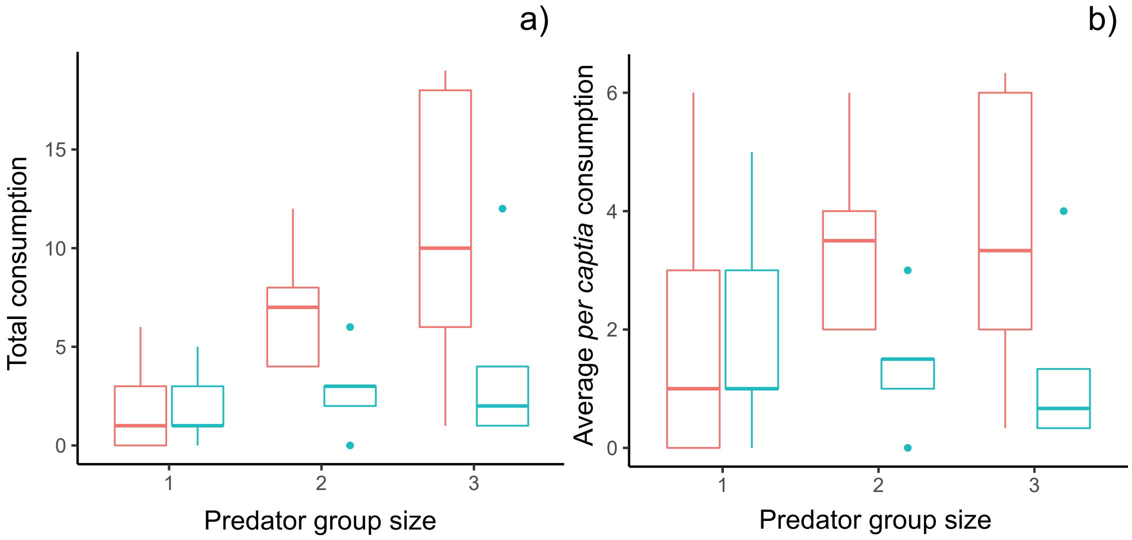

Figure 5. Total consumption (**a**) and average *per capita* consumption (**b**) of *Anentome helena* across three predator group size treatments towards *Physella acuta* at 22 °C (red) and 18 °C (blue) temperature treatments. Trials lasted 48 hours.

Physella acuta activity

Though higher at 22 °C, there was no significant effect of temperature on *P. acuta* activity (mean ± 95% CI: 22 °C, 7.50 ± 3.889; 18 °C, 4.75 ± 1.810; p = 0.164).

Discussion

The international pet trade is highly diverse, but knowledge gaps exist for many of the species sold, both in terms of taxonomic identification and the potential ecological impacts they might have if released. Temperature remains a crucial barrier to IAS establishment, proliferation and impact (Rahel and Olden 2008; Standfuss et al. 2016; Hesselschwerdt and Wantzen 2018), and with the risk of pet releases or escapes ever-present, there remains a need to establish its effect on the potential impacts of commonly traded species. Here, we show that a readily available mollusc in the German pet trade, *A. helena*, or *Anentome* sp. A (Strong et al. 2017), is capable of surviving and feeding at an ecologically relevant temperature lower than typically recommended for the species, but with reduced predatory capacity.

Functional responses, and maximum feeding rates in particular, have been used effectively as ways of quantifying the ecological impacts of invasive species across abiotic contexts (Dick et al. 2014; Dickey et al. 2021). Here, we demonstrated that at the 18 °C temperature treatment there was a significantly reduced maximum feeding rate relative to the 22 °C treatment for *A. helena* on *P. acuta* prey. The functional response method is ultimately a *per capita* measure of impact, however, and while a valuable and popular impact assessment method, it may give a reduced or enhanced consumption rate by often failing to account for interactions between conspecifics. Indeed, conspecific interactions, both synergistic and agonistic, have been shown to facilitate invasive species success. For example, aggression towards conspecifics is thought to facilitate spread (e.g. aggressive individuals inhabiting the range frontier: Groen et al. 2012), while a lack of aggression towards conspecifics may facilitate coexistence in high densities in the invaded range (e.g. Argentine ant, *Linepithema humile* Mayr, 1868: Suarez et al. 1999; Asian shore crab, *Hemigrapsus sanguineus* De Haan, 1835: Hobbs et al. 2017). Further, simply the presence of conspecifics in feeding trials may limit stress and give more representative feeding rates. We therefore incorporated group feeding trials into our study, with different predator densities (i.e. foraging group sizes) and fixed prey densities in order to assess the role of temperature and group size on the total feeding rates and average *per capita* feeding rates (defined as total consumption divided by group size). For the latter measure, we propose that consistent feeding rates across group sizes would indicate neutral interactions, whereas increasing would be indicative of synergistic interactions and decreasing indicative of agonistic interactions. In our trials, temperature and group size were shown to significantly affect total consumption, however, there was a lack of significant two-way interaction, indicating that group size played the same role on consumption rates at both temperatures. While there was an effect approaching significance of temperature on average *per capita* feeding in the group trial, the lack of predator density effect suggests neutral rather than synergistic (for example, coordinated group hunting) or agonistic group feeding effects (such as interference competition).

While not significant between the two temperature treatments, prey activity levels were higher at 22 °C, which could potentially have increased the encounter rate and in turn led to a higher attack rate, but this turned out not to be the case, with no significant difference in attack rates between the two treatments. However, maximum feeding rates were significantly different, and this may have been driven by the predator's metabolism and digestion (Jeschke et al. 2002; Brown et al. 2004;

but see Marshall and McQuaid 2011) or handling time. Hunting by *A. helena* involves subduing prey with their foot, and then consuming it via its aperture (Berkhout and Morozov 2022). It may be that hunting or digesting prey became more difficult, or more protracted processes at the colder treatment. Considering the 48-hour experiment time, digestion may have had the stronger influence on observed consumption, thus the observed differences are probably due to a longer digestion time at the colder treatment. This may have long-term implications for survival of the species at more stressful temperatures. We recommend future studies to assess the role of temperature on alternative, less energetically costly food resources, such as less motile or less protected prey, or on carrion which they are also known to feed upon (Bogan and Hanneman 2013). Indeed, a switch from hunter to scavenger at lower temperatures may facilitate survival. Studies assessing the role of various abiotic stressors on *A. helena* mortality would prove useful too (e.g. Paiva et al. 2020; Cuthbert et al. 2021), such as lower temperatures, differing dissolved oxygen levels and salinity levels representative of estuarine conditions or urban ponds (Van Meter et al. 2011). Further, as a dioecious gastropod (Coelho et al. 2013), unlike invasive species exhibiting parthenogenesis such as *M. tuberculata* (Brande et al. 1996) and *Tarebia granifera* (Miranda et al. 2011), the presence of both sexes is crucial to the establishment of wild populations. How such abiotic conditions affect reproduction and whether there are survival differences between sexes require further study.

In the short term, it appears likely that the areas most at risk from *A. helena* are thermally influenced freshwater system, of which there are many in Europe. The Rhine, the Weser and the Po watersheds have all been shown to have a large proportion of their flows affected by thermal pollution during the year (Raptis et al. 2016). The Gillbach-Erft river system in Germany, for example, with temperatures rarely falling below 19 °C, became the home of numerous tropical and subtropical fish, crustaceans and molluscs (Emde et al. 2016; Jourdan et al. 2017; Lukas et al. 2017), and thermal pools in Hungary have also seen the establishment of diverse, non-native crayfish assemblages (Weiperth et al. 2020). Further, climate change as well as expanding urban areas with heat island effects – deemed “gateways to new ranges” (Borden and Flory 2021) – are of particular note considering that this is where most pet trade releases occur. Further research is required focused on how such habitats might host populations capable of undergoing inherent or prolonged lags (Crooks 2005), or “sleeper populations” waiting for a trigger to become abundant and disruptive (Spear et al. 2021), and facilitate adaptation and spread to colder conditions. Going forward, we propose that combining the monitoring of pet stores, garden centres, websites and online marketplaces (Ng et al. 2016b; Shivambu et al. 2020a, 2020b; Olden et al. 2021), with methods to assess the potential impacts of the species sold (Dickey et al. 2022) and their “hitchhikers” (Stanicka et al. 2022), will prove increasingly crucial in helping to determine the next potential invaders.

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Author’s contribution

JWED, JMJ and EB conceived the study. JWED performed the experiments, with valuable assistance from GTS, and performed statistical analysis and prepared the initial manuscript. EK conducted molecular laboratory work, while RSB processed the molecular data. All authors provided valuable input to the development of the final manuscript and have given approval for publication.

Data availability

Sequence data used for species identification is available on NCBI accession number OQ075971. Accessions for all samples used in this study can be found in Suppl. material 1: table S1.

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Supplementary material 1

Additional materials

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Data type: tables and figure (docx file)

Explanation note: table S1: Sample information downloaded from NCBI to allow us to validate the identification of our *A. helena* study organisms ("Our sample"). Known samples representing the four cryptic species (see Strong et al. 2017) as well as the closely related *Nassodonta dorri* and *Naytia granulosa* as an outgroup were used; table S2: First-order terms, FR parameters and associated significance levels resulting from logistic regression of proportional prey consumption across different prey densities at two experimental temperature treatments (22 °C and 18 °C) for the predator *Anentome helena* feeding on *Physella acuta* prey over 48 hours. The significant negative first-order term values under both temperature treatments indicate Type II functional responses (cf. Pritchard et al. 2017). Attack rate (a) and handling time (h) parameter estimates derived using Rogers' random predator equation (Eq. 1), with p values obtained from the "frair_fit" function (Pritchard et al. 2017) indicative of estimate reliability. '**' $p < 0.01$. '*' $p < 0.05$; table S3: Comparisons of fitted coefficients using the delta or difference method of Juliano (2001) as provided by the frair package (Pritchard et al. 2017). Significant differences emboldened; table S4: Tukey post-hoc comparisons between total consumption of *Physella acuta* prey by *Anentome helena* across group size - temperature treatments; figure S1: Experimental water bath set-up featuring arenas (1 L plastic jars filled with 400 ml of water), air lines (drawn from a larger, orange tube), a heater and probe, and two circulation pumps to ensure uniform distribution of temperature, as demonstrated by photographs a) and b), and schematic c).

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