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Comparative feeding rates of native and invasive ascidians

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ABSTRACT

Ascidians have a recent history of species introductions globally, often with strong ecological impacts. Comparisons of per capita effects of invaders and comparable natives are useful to assess such impacts. Here, we explore ingestion rates (IR) and clearance rates (CR) of *Ciona intestinalis* and *Ciona robusta*, co-occurring native and non-native ascidians, respectively, from Brittany, France. IR was positively related to food concentration, with the invader responding more strongly to increasing food concentration. CR also differed by species, with the invader demonstrating higher values. *C. robusta* exhibited a higher functional response (Type I) than did *C. intestinalis* (Type II). Relative impact measured using seasonal abundance and IR revealed that *C. robusta* has a much greater impact than *C. intestinalis* at all food concentrations tested, though the former has a constrained distribution which limits its regional impact. Nevertheless, when abundant, we expect *C. robusta* to exert a greater impact on algal foods.

1. Introduction

Aquatic invasive species (AIS) are increasingly common in both marine and freshwater habitats world-wide owing to a combination of intentional (e.g. stocking) and unintentional (e.g. hull fouling, ballast water) introductions (e.g. Ruiz et al., 2000; Ricciardi, 2006). AIS are among the strongest stressors in many aquatic ecosystems (e.g. Clavero and García-Berthou, 2005; Allen et al., 2013; Arthington et al., 2016), with a subset of introduced species exerting strong ecological, health and/or economic costs.

Ascidians are primarily sessile organisms with a brief pelagic (lecithotrophic) larval stage. Numerous ascidian species have experienced an increase in global range linked to human-mediated spread and, in some cases, climate warming (e.g. Lambert, 2001, 2007; Sorte et al., 2010; Bock et al., 2011; Zhan et al., 2015; Simkanin et al., 2016; Nydam et al., 2017). In total, Zhan et al. (2015) catalogued 80 species that were recognized as non-native in the habitats in which they were reported. In the Netherlands, non-native ascidians colonized during two major spates, one in 1974–1977, the other in 1991–2004 (Gittenberger, 2007). The increase in the number of non-native ascidian species reported in the USA was low but relatively linear between 1850 and 1950, thereafter increasing sharply on both Pacific and Atlantic coasts (Simkanin et al., 2016).

On the English Channel coast of Brittany, France, non-native *Ciona robusta* (previously *Ciona intestinalis* type A) co-occurs with *C. intestinalis* (previously *C. intestinalis* type B) (Brunetti et al., 2015; Bouchemousse et al., 2016). While the arrival date of *C. robusta* is not known, it is believed to have established since the turn of the 21st century (Bouchemousse et al., 2016). As the species occupy the same habitats and are filter feeders, they have the potential to compete for settling substrates or for food (Bouchemousse et al., 2017).

Human-mediated range enhancement of ascidians has been effected mainly by fouling on ships, transfer on equipment or as a fellow traveler on aquaculture stock, and by fishery or recreational boats, although there is a small likelihood of transfer in ballast water (see Zhan et al., 2015). As introduced ascidians often adversely affect recipient communities, their spread is cause for concern (see Lambert, 2009; Zhan et al., 2015).

Identifying which introduced species are likely to produce strong impacts is a daunting challenge owing to the varying nature of the

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species themselves, the nature of the ecosystems that they are introduced into, and a variety of context-dependencies (Kumschick et al., 2015). Recently, Dick et al. (2014) proposed analyzing comparative functional responses (FR) of introduced species (or those that might be introduced) versus those of comparable native taxa to assess whether the former would have high impact. The functional response considers a species' per capita resource consumption as a function of resource availability, and Dick et al.'s (2014) study highlighted that invader FRs typically exceed those of native species. This approach was then extended by combining it with species abundance data to yield a total impact potential for the invader scaled to that of the native species (Dick et al., 2017a).

In this study, we explore the comparative feeding ecology of non-native *C. robusta* and native *C. intestinalis* to determine whether these taxa have similar feeding attributes and expected ecological effects, or whether the general pattern of invader > native that Dick et al. (2014) identified also holds for these very similar ascidians.

2. Methodology

2.1. Sampling procedure

Ciona individuals were collected by scraping from pontoons and pillars in Brest, France on 25th September 2015 and acclimated in the animal husbandry lab (18 °C) at the Roscoff Biological Station, France. *Ciona* cultures were maintained on *Isochrysis affinis galbana* algae (issued from the Roscoff Culture Collection (RCC) facility under the reference numbers RCC1349) at concentrations of 15–20 × 10^6 cells/mL. Seven experimental food concentration treatments (1508, 3380, 5900, 12,873, 29,539, 51,616, 133,084 cells/mL) were determined using a Malassez cell counting chamber. Experimental *Ciona* individuals were housed in separate cylindrical tanks, each filled with 2 L seawater, totaling seven tanks. Six of the seven tanks contained *Ciona* individuals, with the remaining one containing only *Isochrysis affinis galbana*, thereby serving as a control. Of the six experimental tanks, three contained a single *C. intestinalis* individual and food, while the other three had a single *C. robusta* individual and food. *Ciona* individuals were randomly selected and distinguished morphologically. This design was repeated twice to produce six replicates for each ascidian species at each algal concentration, with the exception of the treatment with 12,783 algal cells/mL, where time constraints only allowed for three replicates of each species.

2.2. Experimental setup

At the beginning of each trial, individuals were placed upright at the midpoint of each tank, while 10 mL of concentrated algae suspension was injected into the centre of the tank. The algal suspension was mixed using an air bubbler affixed with plasticine to the side of each tank. Feeding trials were run for 1 h, after which algal cell counts were obtained using flow cytometry, focusing on cells 3.5–6 μm in diameter (Bendif et al., 2013). Three replicate 1.5 mL samples were collected from the centre of each tank (2 cm below the surface) to assess algal concentration, and stored in 1.6 mL Eppendorf tubes containing 15 μL of 25% glutaraldehyde at −80 °C for preservation. Cell densities were then quantified using a Cell Lab Quanta Flow Cytometer (Beckman Coulter, Inc.) at a calibrated flow rate of 30 μL min^{-1}, and cell counts were converted to cells/mL. Final and initial algal concentrations in experimental tanks were compared with a correction for controls lacking animals. Following the experiment, the middle gut (from the stomach to the anus) of each individual was excised using 10% bleach-sterilized razor blades to ensure the mass of ingested food was excluded from subsequent body mass measurements. Individuals were then desiccated in an oven at 65 °C to obtain total dry weight (g), which included a previously measured cup weight. Cup weight was subtracted from this value to obtain the dry weight of each individual.

2.3. Statistical analyses

The Ingestion Rate (IR) of each individual was measured using both pre- and post-experiment algal cell counts, adjusted for controls, as:

$$IR = \frac{[(E_0 - E_t) - (C_0 - C_t)]}{t}$$

where E_0 and E_t represent experimental algal cell concentrations at times 0 and t, respectively, C_0 and C_t represent control algal cell concentrations at times 0 and t, and t is experimental duration (1 h). $C_0 - C_t$ was included to adjust temporal changes in algal concentration due to algal growth or sedimentation in controls. Pre- and post-experiment algal concentrations were also used to determine the Clearance Rate (CR) of each individual as:

$$CR = V\frac{[\ln(E_0/E_t) - \ln(C_0/C_t)]}{t}$$

where V represents the volume of suspension. As with IR, a correction factor was included in CR to account for changes in control tanks where no animals were present (Coughlan, 1969).

Four of six calculated IR and CR values for *C. intestinalis* at the highest algal concentration (133,084 cells/mL) were negative, possibly owing to a combination of sinking algal cells and/or less active animal feeding. Consequently, we removed this algal density from analysis for both species. Seven other negative values for IR and CR were also found for *C. intestinalis* and subsequently discarded when performing analysis. The reason for this problem is not clear, as we attempted to minimize disturbance and stress on animals prior to all feeding trails. We conducted two three-way ANOVA tests with the factors species, animal mass, food density, and their interactions to determine which factors affected IR and CR. Statistical analyses were performed in R-3.5.0 (R Core Team, 2018) and RStudio 1.1.447 (RStudio Team, 2016).

Animal functional response to varying food concentration was modeled using the FRAIR package in R-3.5.0 (Pritchard, 2017). As food was not replaced after consumption, Rogers' random predator equation was used to describe the type II functional response of *C. intestinalis* (Barrios-O'Neill et al., 2014; Rogers, 1972):

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

where N_e is the number of food items consumed, N_0 is the initial concentration of algal cells, a is attack rate, h is handling time, and T is experimental duration. As the ingestion rate of *C. robusta* generally increased linearly with increasing food concentration, a type I fit was applied to the consumption data (FRAIR; Pritchard, 2017):

$$N_e = N_0(aT)$$

These models were then non-parametrically bootstrapped (n = 2000) to generate 95% confidence intervals for the functional response curves.

Relative Impact Potential (RIP; Dick et al., 2017a) was calculated for co-occurring ascidians using relative field abundance data from Brittany, France. For each instance in which the species co-occurred (130 in total; Bouchemousse et al., unpublished data, see Bouchemousse et al. (2017) for the sampling protocol) we obtained the ratio of relative abundance (A; measured as Ind./m^2) of *C. robusta* to *C. intestinalis*. We then randomly drew (from between three and six measures per species) an IR for the invader and another for the native species. Relative Impact Potential (RIP) was then estimated as the product of a randomly drawn A (from 130 co-occurrence cases) and a randomly drawn IR ratio (invader IR divided by native IR) for that food concentration. Results were bootstrapped 10,000 times incorporating different combinations of A and IR. Similar calculations were repeated for each food concentration. In Dick et al. (2017a)'s original formulation of RIP, abundance was measured directly and utilized maximum feeding rate ($1/h$) from functional responses. Any combination of A and IR that yields a RIP > 1 indicates a greater relative impact by the non-