**QUEEN'S
UNIVERSITY
BELFAST**

Using functional responses and prey switching to quantify invasion success of the Pacific oyster, *Crassostrea gigas*

Joyce, P. W. S., Dickey, J. W. E., Cuthbert, R. N., Dick, J. T. A., & Kregting, L. (2019). Using functional responses and prey switching to quantify invasion success of the Pacific oyster, *Crassostrea gigas*. *Marine environmental research*. <https://doi.org/10.1016/j.marenvres.2019.02.010>

Published in:
Marine environmental research

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights

Copyright 2019 Elsevier Ltd.

This manuscript is distributed under a Creative Commons Attribution-NonCommercial-NoDerivs License

(<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Accepted Manuscript

Using functional responses and prey switching to quantify invasion success of the Pacific oyster, *Crassostrea gigas*

Patrick W.S. Joyce, James W.E. Dickey, Ross N. Cuthbert, Jaimie, T.A. Dick, Louise Kregting

PII: S0141-1136(19)30022-4

DOI: <https://doi.org/10.1016/j.marenvres.2019.02.010>

Reference: MERE 4684

To appear in: *Marine Environmental Research*

Received Date: 8 January 2019

Revised Date: 13 February 2019

Accepted Date: 17 February 2019

Please cite this article as: Joyce, P.W.S., Dickey, J.W.E., Cuthbert, R.N., Dick, J., T.A., Kregting, L., Using functional responses and prey switching to quantify invasion success of the Pacific oyster, *Crassostrea gigas*, *Marine Environmental Research* (2019), doi: <https://doi.org/10.1016/j.marenvres.2019.02.010>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Using functional responses and prey switching to quantify invasion success of the Pacific
oyster, *Crassostrea gigas*

Patrick W.S. Joyce^{1,2*}, James W.E. Dickey², Ross N. Cuthbert², Jaimie, T.A. Dick², Louise
Kregting^{1,2}

¹School of Natural and Built Environment, Queen's University Belfast, Queen's Marine
Laboratory, 12-13 The Strand, Portaferry, BT22 1PF, UK

²Institute for Global Food Security, School of Biological Sciences, Queen's University
Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK

*Corresponding author email: pjoyce04@qub.ac.uk

Abstract

Invasive alien species continue to proliferate and cause severe ecological impacts. Functional responses (FRs) have shown excellent utility in predicting invasive predator success, however, their use in predicting invasive prey success is limited. Here, we assessed invader success by quantifying FRs and prey switching patterns of two native predators, the common sea star, *Asterias rubens*, and the green crab, *Carcinus maenas*, towards native blue mussels, *Mytilus edulis*, and invasive Pacific oysters, *Crassostrea gigas*. *Asterias* displayed destabilising type II FRs, whereas *Carcinus* displayed stabilising type III FRs towards both prey species. Both predators exhibited greater search efficiencies and maximum feeding rates towards native compared to invasive prey. Both predators disproportionately consumed native mussels over invasive oysters when presented simultaneously, even when native mussels were rare in the environment, therefore indicating negligible prey switching. We demonstrate that invasion success may be mediated through differential levels of biotic resistance exerted by native predators.

Keywords

Biotic resistance; Crabs; Frequency dependent predation; Invasive species; *Per capita* consumption; Predation; Sea stars

Introduction

The rate of invasive alien species (IAS) introductions is increasing (Seebens et al. 2017, 2018). Many IAS can drive changes in biodiversity (Molnar et al. 2008), habitat (Burlakova et al., 2012) and community structure (Sanders et al. 2003, Guy et al. 2018), often leading to novel species interactions between natives and invaders (Skein et al., 2018). Previously, there has been a sustained focus on the effects of invasive predators on native prey (Dick et al. 2017), with invasive predators perceived to have greater impacts on communities than invasive prey (Salo et al. 2007). The latter can, however, establish in large numbers with the potential to displace native species (Burlakova et al., 2000) and alter predator-prey and competitive interactions (Waser et al., 2015). Predicting the outcomes of such species introductions has been elusive and plagued with contradictory meta-analyses of species traits and lack of application of appropriate methods (Dick et al. 2014).

Functional responses (i.e. resource use as a function of resource density), and prey switching (“frequency dependent predation”), are well known, fundamental ecological concepts, however, invasion ecologists have been slow to capitalise on their use in assessing and predicting ecological impacts of invasive species. Comparative functional response (FR) analysis has shown excellent utility in assessing and predicting invader impacts (Dick et al. 2014, 2017, Lavery et al. 2017), but with little application to biotic resistance (Twardochleb et al. 2012). Comparative FRs achieve this because they quantify the relative magnitude of invader *versus* native equivalent *per capita* effects, that then translate into population consequences for native resources such as prey species (Dick et al. 2017). Prey switching may complement FR methods (Cuthbert et al. 2018b), as both approaches can inform population level outcomes of species interactions. Prey switching by predators can impact prey population stability by controlling abundant prey species while simultaneously providing refuge for scarce prey (Murdoch 1969). Thus, if invading prey are consumed by

native predators that can readily switch between resources, then some level of biotic resistance toward the invader may occur (Sousa et al., 2009; Carlsson et al., 2011). Conversely, predators that do not exhibit such switching behaviour may reduce the abundance of one prey type while allowing the increase of another. Thus, in situations where invasive prey are consumed at a lesser rate than native prey, or are ignored completely, the reduced predation pressure on invasive prey along with continuous removal of native competitors is likely to exacerbate the invasion (Cuthbert et al. 2018a,b). Although this concept appears elementary, there is a distinct lack of investigation into prey switching, especially with regards to invasive species.

The Pacific oyster, *Crassostrea gigas*, is one of the most successful invertebrate invaders worldwide (Ruesink 2007, Herbert et al. 2016) and has been documented to colonise and flourish on beds of the native blue mussel, *Mytilus edulis* (e.g. Kochmann et al. 2008). Beds of *M. edulis* that are invaded by *C. gigas* can experience a shift in the dominant ecosystem engineer (Kochmann et al. 2008, Reise et al. 2017a) which may lead to implications for the native community. Not only has oyster reef formation been suggested to alter *M. edulis* population size structure by reducing predation on small mussels (van der Zee et al. 2012), they can also negatively affect foraging in higher trophic levels (Markert et al. 2013). However, invasions by Pacific oysters have not led to catastrophic declines in native species and complete dominance of benthos (Reise et al. 2017b) as has been the case with, for example, zebra mussels in many freshwater ecosystems (Ricciardi et al. 1996, 1998). Thus, we expect to find evidence that Pacific oysters suffer some degree of biotic resistance from native species, particularly by predators such as crabs owing to their generalist feeding nature (Walne & Davies 1977).

Blue mussels provide an important and abundant food resource for a range of intertidal and subtidal predators including birds, sea stars, and crabs (Ebling et al. 1964;

Paine, 1974; Nehls et al., 1997). Throughout Europe, two of the main invertebrate predators of *M. edulis* are the common sea star, *Asterias rubens*, and the European green crab, *Carcinus maenas* (Dolmer, 1998; Murray et al., 2007). Seasonal swarming of *A. rubens* onto beds of *M. edulis* has the potential to destroy local populations (Dare 1982) and *C. maenas* has been found to have serious impacts on commercially valuable mussel beds (Murray et al., 2007). Although the diets of *A. rubens* and *C. maenas* often contain mussels, they are considered generalist predators (Miron et al., 2005). This generalist feeding nature suggests an ability to switch between prey species, or show strong preferential feeding on some species, depending on relative abundances available. Mascaró & Seed (2001) observed consumption of *C. gigas* by *C. maenas* thus, these native predators may potentially exert some level of biotic resistance toward *C. gigas*, which may reduce invasion success, but also reduce competitor abundance, hence aiding invader success.

Here, we thus employ functional response and prey switching experiments to identify density-dependent predation rates and prey switching/preference of predators towards both invasive and native prey, in isolation and when presented simultaneously. Using two dominant native predators, the common sea star, *Asterias rubens*, and the green crab, *Carcinus maenas*, we aimed to investigate predation of the native blue mussel, *M. edulis*, and the Pacific oyster, *C. gigas*, and the balance of predator driven biotic resistance or facilitation of *C. gigas*.

Methods

Animal collection and maintenance

Common sea stars, *A. rubens*, were collected by hand from shallow waters in Strangford Lough by snorkelling whilst green crabs, *C. maenas*, were collected using baited crab pots from a rocky shore within 50 m of the sea star collection site (54° 23' 30" N, 05° 34' 29" W). Animals were maintained at Queen's Marine Laboratory, Portaferry in through-

flowing, sand filtered seawater ($13^{\circ}\text{C} \pm 1^{\circ}\text{C}$) pumped from the adjacent Strangford Lough. Sea stars were fed whole mussels (> 40 mm shell length) *ad libitum* and held in uncovered, ~500 L tanks allowing natural light conditions for two weeks prior to feeding trials. All sea stars were size matched for experimental use with maximum arm lengths measuring 70 – 90 mm. Green crabs were fed raw herring every two to three days and were held under similar conditions to the sea stars for a minimum of one week prior to experiments. Male crabs were selected to avoid confounding effects of sex and size matched with respect to carapace width (60-70 mm) to minimise variations due to sex and size. Only individuals free of parasites and with both claws intact were used in the experiments. Prior to feeding trials, both sea stars and green crabs were starved for 48 hours to standardise hunger levels. Survivability of all predators were monitored in the laboratory for at least one week after the experiments to ensure all individuals were healthy at the time of feeding. Sea star and green crab experiments were conducted in June 2018 and August 2018, respectively.

The prey, juvenile native blue mussels, *Mytilus edulis*, were collected by hand from a local rocky shore ($54^{\circ} 28' 13''$ N, $05^{\circ} 32' 26''$ W), and juvenile invasive Pacific oysters, *Crassostrea gigas*, were obtained commercially (Guernsey Sea Farms Ltd, Guernsey). Prey were maintained under the same conditions as predators. Prey animals used in feeding trials had shell lengths of 15 – 20.99 mm, a size which is most often consumed by crabs with carapace widths used here (Mascaró & Seed, 2001) and to avoid any confounding effects of prey size. Although *A. rubens* may prefer larger prey than offered here (Saier 2001) this is likely driven by refuge provision. Dolmer (1998) however, observed no size selection of prey by *A. rubens* when offered in isolation.

Feeding trials

Functional responses (FRs)

For sea stars, feeding trials were conducted in $36 \times 26 \times 18$ cm arenas filled with 15 litres of continuously aerated, sand filtered seawater pumped from the adjacent Strangford Lough, to which the sea stars were previously acclimated. For the FRs, predators were presented with either native mussel or invasive oyster prey at six densities (1, 2, 4, 8, 15 or 30; $n = 4$ per experimental group) which were added to each of the arenas five minutes before the addition of a predator. Trials started at 09:00 and lasted 48 hours, after which predators were removed and remaining live prey counted. Due to the number of sea stars ($n = 40$) necessary for the experiment ($n = 48$ trials), individuals were reused until sufficient replication was complete. Individuals were not exposed to the same treatment twice to avoid pseudoreplication.

For green crabs, feeding trials were conducted in $56 \times 36 \times 23$ cm arenas covered with plastic mesh (1 cm mesh size) to allow natural light to penetrate but prevent crabs from escaping. Running sea water, pumped from the adjacent Strangford Lough, was delivered to the arenas via hoses supplying water at a rate of $\sim 1.5 \text{ L min}^{-1}$. For the FRs, predators were presented with either native mussel or invasive oyster prey at six densities (2, 4, 8, 16, 32 or 64; $n \geq 3$ per experimental group). Native mussels naturally attached to the base of the arena with byssus threads and invasive oysters were adhered to the base of the arena with a small amount of epoxy putty to simulate natural attachment. Attachment of prey to the bottom of experimental arenas was necessary for feeding trials with green crabs to ensure prey were not washed away or moved by the running water. Prey animals were added to each of the arenas 13 hours prior to the feeding period. For consistency, trials started at 09:00 and lasted 8 hours, after which predators were removed and the remaining live prey counted. For both predators, controls to capture background mortality of prey included one replicate of each experimental group in the absence of a predator.

Prey switching

Predators from FR experiments were reused for prey switching experiments after a 48-hour starvation period. Switching experiments were performed under the same conditions as the FRs for each of the two predators. For both prey switching experiments, native mussels and invasive oysters were added simultaneously in five different ratios (0:20, 5:15, 10:10, 15:5, 20:0; sea stars, $n \geq 7$ replicates per experimental group; green crabs, $n \geq 5$ replicates per experimental group). Sea stars were allowed to feed for 24 hours with consumed prey being replaced after 12 hours to maintain nominal prey ratios. Green crabs were allowed to feed for two hours with consumed prey being replaced every 15 minutes to maintain nominal prey ratios. For both predators, controls to capture background mortality of prey included one replicate of each experimental group without a predator.

Statistical analyses

All statistical analyses were undertaken in 'R' version 3.4.2 (R Development Core Team 2017).

Functional responses

Overall prey consumption in FR experiments with respect to the factors 'prey species' and 'prey density' for each predator type was compared using generalised linear models assuming Poisson distributions and log links as counts were not overdispersed in relation to degrees of freedom.

Functional response analysis was undertaken using the 'frair' package (Pritchard et al. 2017). Logistic regression considering the proportion of prey consumed as a function of initial prey density was used to determine FR types. A type II FR is determined categorically by a significantly negative first order term and a type III by a significantly positive first order term followed by a significantly negative second order term. Akaike Information Criterion (AIC) was applied to select FR models which minimised information loss. We fit flexible FR models for non-replacement of prey (Real 1977, Barrios-O'Neill et al. 2016):

$$N_e = N_0(1 - \exp(bN_0^q(hN_e - T)))$$

where N_e is the number of prey eaten, N_0 is the initial prey density, b is the search coefficient, q is the scaling component, h is the handling time, and T is the experimental time.

Categorically, type II FRs are indicated when $q = 0$, and when $q > 0$ the FR becomes increasingly sigmoidal. Here, when type II FRs were indicated, q was fixed at 0 and when type III FRs were indicated, q was fixed at 1. The *Lambert W* function was implemented to fit the models to the data (Bolker 2008). Non-parametric bootstrapping ($n = 2000$) was used to produce 95 % confidence intervals (CIs) around the FR curves (Pritchard et al. 2017), enabling results to be considered at the population-level and thus differences to be inferred on the basis of CI overlaps.

Prey switching

In prey switching trials, prey ratios of 5:15, 10:10 and 15:5 were formally included in analyses to omit singularities in prey choice from analyses (0:20 and 20:0). Overall consumption was examined using generalised linear mixed effects models using the package ‘lme4’ (Bates et al. 2015), with ‘proportion available’ integrated as a between factor and ‘prey species’ as a within factor.

Chesson’s selectivity index was used to assess prey preference of sea stars and green crabs separately towards invasive oysters (Chesson 1978, 1983):

$$\alpha_i = \frac{(r_i/p_i)}{\sum_{j=1}^m (r_j/p_j)}$$

where r_i is the proportion of prey type i in the diet, p_i is the proportion of prey type i available, m is the number of prey types, r_j is the proportion of prey type j in the diet, p_j is the proportion of prey type j available. The value of α_i ranges from 0 to 1 with $\alpha_i > 0.5$ ($1/m$) inferring positive preference, $\alpha_i < 0.5$ inferring negative preference, and $\alpha_i = 0.5$ inferring no

preference for either prey type. Chesson's selectivity indices were transformed to reduce extremes (0s and 1s):

$$\alpha_t = \frac{\alpha_i(n-1) + 0.5}{n}$$

where α_t is the transformed output and n is the sample size. Beta regression was then used to compare observed α_t values towards invasive oysters with predicted values under no preference (i.e. 0.5) across proportions available separately for each predator type using the 'betareg' package in R (Cribari-Neto & Zeileis 2010).

Results

Functional Responses

Prey survival in controls of both FR experiments was > 99 % thus indicating that prey were healthy and mortality in all experimental groups could be attributed to predation.

Figure 1. Functional responses of a) the common sea star, *Asterias rubens* and b) the green crab, *Carcinus maenas*, toward invasive oysters, *Crassostrea gigas* (red), and native mussels, *Mytilus edulis* (blue), with bootstrapped ($n = 2000$) 95 % CIs. Note difference in axes scales.

For sea stars, overall, significantly more native mussels were consumed than invasive oysters ($\chi^2 = 40.82$, $df = 1$, $p < 0.001$) and significantly more prey were consumed when greater densities were supplied ($\chi^2 = 173.12$, $df = 5$, $p < 0.001$). A significant ‘prey species $\times$ prey density’ interaction ($\chi^2 = 27.13$, $df = 5$, $p < 0.001$) reflected greater consumptive differences in favour of native mussels at higher prey densities (Fig. 1a).

Logistic regression revealed that sea stars exhibited type II FRs towards native mussels and invasive oysters (Table 1). Sea stars exhibited a significantly higher FR towards native mussels than towards invasive oysters, with CIs divergent across most prey densities (Fig. 1a). The search coefficient, b , was higher toward native mussels than invasive oysters (Table 1) and handling time, h , tended to be lower toward native mussels (Table 1). Maximum feeding rates, $1/h$ (i.e. FR curve asymptote), were thus higher when consuming native mussels as compared to invasive oysters (Table 1, Fig 1a).

Overall, green crabs consumed significantly more native mussels than invasive oysters ($\chi^2 = 17.37$, $df = 1$, $p < 0.001$) and consumption increased under greater prey densities ($\chi^2 = 463.34$, $df = 5$, $p < 0.01$). A significant ‘prey species $\times$ prey density’ interaction ($\chi^2 = 27.13$, $df = 5$, $p < 0.01$), reflected increased consumption of native mussels compared to invasive oysters under low-intermediate prey densities which is mirrored in the FR (Fig. 1b).

For green crabs, results of logistic regression were equivocal between types II and III (Table 1), however, AIC indicated the type III model was the best fit. Green crabs exhibited a type III FR toward invasive oysters. Functional response CIs were divergent at low prey densities but converged above densities of 20 (Fig. 1b). As with sea stars, the search coefficient, b , was higher towards native mussels than invasive oysters (Table 1) and the handling time, h , of native mussels was lower compared to invasive oysters (Table 1). Maximum feeding rates, $1/h$, therefore tended to be higher towards native mussels (Table 1; Fig. 1b).

Table 1. Results of logistic regression for both type II and type III models of the common sea star, *Asterias rubens*, and the green crab, *Carcinus maenas*, towards invasive oysters, *Crassostrea gigas*, and native mussels, *Mytilus edulis*, considering prey eaten as a function of prey density. Terms marked * indicate significant terms. Parameter estimates resulting from flexible FR models are shown.

	<i>Asterias rubens</i>		<i>Carcinus maenas</i>	
Prey species	<i>C. gigas</i>	<i>M. edulis</i>	<i>C. gigas</i>	<i>M. edulis</i>
Logistic regression				
Type II FR				
First term	-0.029	-0.041	-0.004	-0.036
<i>P</i> value	0.026*	0.006*	0.323	< 0.001*
Type III FR				
First term	-0.181	-0.104	0.070	0.122
<i>P</i> value	0.016*	0.265	0.002*	< 0.001*
Second term	0.004	0.002	-0.001	-0.002
<i>P</i> value	0.040*	0.489	< 0.001*	< 0.001*
Parameter estimates				
<i>b</i>	0.703	2.046	0.077	0.273
<i>h</i>	0.087	0.026	0.036	0.027
<i>q</i>	0	0	1	1
1/ <i>h</i>	11.494	38.760	30.769	37.175

Prey switching

In sea star switching trials, overall, consumption of native mussels was greater than invasive oysters ($\chi^2 = 10.55$, $df = 1$, $p < 0.01$), and prey proportions available did not significantly influence consumption ($\chi^2 = 5.53$, $df = 2$, $p > 0.05$). However, a significant ‘prey species $\times$ proportion available’ interaction ($\chi^2 = 9.53$, $df = 2$, $p < 0.01$) reflected greater consumptive differences in favour of native mussels when under equal prey proportions with invasive oysters.

Chesson’s selectivity indices toward invasive oysters were found to be significantly lower than the null preference value of 0.5 ($\chi^2 = 22.29$, $df = 1$, $p < 0.001$), thus indicating positive preference for native mussels overall (Table 2, Fig 2). This effect was consistent

across all proportions as there was no significant ‘prey species $\times$ proportion available’ interaction ($\chi^2 = 2.19$, $df = 2$, $p = 0.33$), reflecting a lack of prey switching and consistent disproportionate consumptive preference for native mussels over invasive oysters by sea stars (Fig 2).

Overall, green crabs consumed significantly more native mussels than invasive oysters ($\chi^2 = 10.48$, $df = 1$, $p < 0.001$). Consumption tended to be greater when prey were available in greater proportions, but this effect was not significant ($\chi^2 = 2.85$, $df = 2$, $p > 0.05$). Consumption rates of each prey species were consistent across prey ratios as the ‘prey species $\times$ proportion available’ interaction was not significant ($\chi^2 = 5.69$, $df = 2$, $p > 0.05$).

Table 2. Chesson’s selectivity indices ($\alpha_i \pm \text{S.E.}$) for the common sea star, *Asterias rubens*, and the green crab, *Carcinus maenas*, toward invasive oysters, *Crassostrea gigas*, and native mussels, *Mytilus edulis*, at varying prey proportion availabilities. Selectivity indices above 0.5 indicate positive preference whilst indices below 0.5 indicate negative preference of a prey type.

Predator:prey combination	Proportion available	Chesson $\alpha_i \pm \text{S.E.}$
<i>Asterias rubens</i>		
<i>C. gigas</i>	0.25	0.19 ± 0.08
	0.50	0.11 ± 0.05
	0.75	0.32 ± 0.13
<i>M. edulis</i>	0.25	0.68 ± 0.13
	0.50	0.89 ± 0.05
	0.75	0.81 ± 0.08
<i>Carcinus maenas</i>		
<i>C. gigas</i>	0.25	0.22 ± 0.07
	0.50	0.44 ± 0.04
	0.75	0.24 ± 0.05
<i>M. edulis</i>	0.25	0.76 ± 0.05
	0.50	0.56 ± 0.04
	0.75	0.78 ± 0.07

292 Chesson's selectivity indices towards invasive oysters were, again, significantly lower
293 than the null preference values of 0.5 overall ($\chi^2 = 31.49$, $df = 1$, $p < 0.001$), thus indicating
294 positive preference of native mussels over invasive oysters by green crabs (Table 2; Fig. 2b).
295 A significant 'prey species $\times$ proportion available' interaction ($\chi^2 = 12.26$, $df = 2$, $p < 0.01$)
296 reflected reduced preference of native mussels when available at proportions of 0.5, but a
297 lack of prey switching was evidenced overall given a disproportionate preference for native
298 mussels across all choices (Fig. 2).

Figure 2. Proportion of either *Crassostrea gigas* (red) or *Mytilus edulis* (blue) consumed (mean $\pm$ S.E.) as a function of their respective proportional availability for a) the common sea star, *Asterias rubens*, and b) the green crab, *Carcinus maenas*. The solid line indicates expected values if no prey preference was to occur. The dashed line indicates a hypothetical prey switching pattern.

Discussion

With the rate of spread of invasive alien species (IAS) showing little sign of abating (Seebens et al. 2018), invasion ecology needs effective methodologies for assessing and predicting the success and impacts of established, emerging, and future IAS (Dick et al. 2017, Cuthbert et al 2018b, Dickey et al. 2018). Functional responses (FRs) have shown excellent utility in predicting the success and impacts of invasive predators (Dick et al. 2014, Bovy et al. 2015, Xu et al. 2016, Lavery et al. 2017), yet few studies examine FRs toward invasive prey (but see Twardochleb et al. 2012). While FRs have been shown to offer good predictive ability of IAS impacts (Dick et al. 2017), the inclusion of prey switching experiments, with more than one prey species provided simultaneously, could further enhance our knowledge (Cuthbert et al. 2018b). This is because FRs in terms of type (i.e. shape of curve) and magnitude (i.e. maximum feeding rates), plus switching (i.e. “frequency dependent predation”), help quantify the population outcomes of consumer behaviour towards resources (e.g. predator: prey, see Dick et al. 2014).

Pacific oysters have invaded ecosystems worldwide but have not had drastic impacts or caused competitive exclusion of other bivalve species (Reise et al. 2017b). To quantify this pattern of invasion we combined FR and prey switching experiments using two native predators, the common sea star, *Asterias rubens*, and the green crab, *Carcinus maenas*, toward the native blue mussel, *Mytilus edulis*, and invasive Pacific oyster, *Crassostrea gigas*. *Asterias rubens* exhibited a higher-magnitude type II FR toward *M. edulis* compared to *C. gigas*, while *C. maenas* showed type III FRs towards both prey species, with similar maximum feeding rates between prey types but increased predation upon native *M. edulis*, particularly at low densities. We also found that when prey were offered simultaneously, both predators disproportionately consumed native *M. edulis* over invasive *C. gigas* prey, even

when *M. edulis* was rare in the environment, thus suggesting a lack of predator-driven biotic resistance towards *C. gigas* invasion.

Although *A. rubens* exhibited destabilising type II FRs towards both prey species, increased search efficiency and decreased handling times were shown toward native *M. edulis* compared to invasive *C. gigas*. Consequently, our results indicate that *A. rubens* exerts strong destabilising impacts toward native *M. edulis* due to high predation rates at low prey densities and lower handling times which drive high maximum feeding rates (Dick et al. 2013), and reduced predation pressure upon invasive *C. gigas*. In particular, lower handling times of *A. rubens* and *C. maenas* towards *M. edulis* compared to *C. gigas* prey suggests that predators are quicker to open, consume, and digest the native prey, which may be a function of shell morphology or thickness, even though shell length between prey species was matched in experiments (Griffiths & Seiderer 1980).

Whilst green crabs displayed greater similarities in maximum feeding rates between prey species, feeding rates were considerably higher towards the native prey. In contrast to sea stars, green crabs displayed equivocal type II and type III FRs toward native *M. edulis* and a more stabilising type III FR toward invasive *C. gigas*. Although unexpected due to the experimental setup, where type II FRs tend to emerge when predators cannot “switch”, type III FRs are likely to be encountered under natural conditions and have previously been found toward mussel prey (Griffen & Delaney 2007). Type III FRs suggest the presence of a low density refuge for prey, reducing the risk of predation when prey fall below a certain threshold (Murdoch & Oaten 1975). The higher search efficiency which *C. maenas* displayed toward native *M. edulis* suggests a greater predatory impact due to a higher rate of prey consumption at low prey densities, which may be more destabilising for native mussel populations at low densities.

For generalist predators such as *A. rubens* and *C. maenas*, invasive prey species may provide a novel resource should they be recognised as prey. Ruesink (2007) found significant predation upon *C. gigas* on rocky shores suggesting these ecosystems exert biotic resistance toward the invasion. Here, although *A. rubens* were offered loose prey and *C. maenas* were offered prey under more ‘realistic’ conditions whereby prey animals were attached to the substratum, when native *M. edulis* and invasive *C. gigas* were provided simultaneously, we found that neither predator showed evidence of prey switching behaviour. Native *M. edulis* was disproportionately consumed by *A. rubens* across all prey species proportion combinations, thus sea stars actively sought native *M. edulis* prey, even when availability was low. Mascaró & Seed (2001) investigated prey choice of *C. maenas* towards several bivalve species, however, the proportional availabilities of the prey species were not altered. Such frequency dependent predation is often overlooked, yet is considered a major driver of genetic, phenotypic, and species diversity (Clarke 1962, Greenwood & Elton 1979, Allen 1988, Whiteley et al. 1997). Species or polymorphs that are rare in the environment may be disproportionately ignored by predators allowing their abundance to increase and *vice versa*, thus substantially contributing to diversity changes. The lack of switching by both predators indicates that even when occupying low proportions of prey availability, native *M. edulis* are consumed at disproportionately high rates. Invasive *C. gigas* are consumed in low numbers irrespective of availability, and are largely relieved from predation pressure, contrary to the alternative prey hypothesis (Angelstam et al. 1984). This low level of predation suggests a small amount of predator driven biotic resistance towards *C. gigas* which corroborates with its limited ecological impacts (Reise et al. 2017b).

Naïveté due to lack of co-evolutionary history is a common concept in invasion ecology however the concept is most often applied to native prey in response to invasive predators (Cuthbert et al. 2018a). Also, it has also been shown that conditioning predators to

prey species can occur (Hall et al. 1982). The predators in this study, however, were collected from a site where *M. edulis* is common whereas *C. gigas* is scarce. The feeding of *M. edulis* to *A. rubens* prior to experimental trials is thus unlikely to have provided any extra conditioning to their natural state. The predators here were thus faced with a novel prey choice and the results provide insights into predation patterns at initial states of *C. gigas* invasion. Although the preference for *M. edulis* over *C. gigas* appeared greater for *A. rubens* compared to *C. maenas*, the lack of significant switching shown by both predators further suggests native predator naïveté towards *C. gigas*, and that laboratory conditioning was unlikely a driver of these results. The preference pattern observed may be because neither predator recognises the novel chemical cue of *C. gigas* as potential prey. Certainly, prolonged exposure of predators to *C. gigas* as an available prey source may alter these predation patterns, however we show that biotic resistance to initial invasions is unlikely. Chemical cues are understood to play several roles in predator-prey dynamics (Weissburg & Zimmer-Faust 1994, Leonard et al. 1999, Griffiths & Richardson 2006) and are commonplace in the aquatic environment. Invasive alien species have been shown to produce chemical cues which may facilitate their invasion by inducing behavioural displacement of native species (Raw et al., 2013). Further investigation into chemical detection of invasive prey by native predators should be investigated as evolutionary experience and learning may increase predation on invasive prey species in the future.

Although the mechanism as to why *C. gigas* consumption by predators is reduced in this study needs further investigation, comparisons of predation towards trophically similar native and invasive prey gives further insights into predator-driven biotic resistance towards invasions. The disproportionate consumption of *M. edulis* over *C. gigas* may facilitate invasions by reducing predation pressure on invasive *C. gigas* and removing potential competitors for resources (i.e. space) from the system. If such patterns were to occur in the

field, then predation pressure may further increase towards *M. edulis* populations. Conversely, it may be predicted that habitat complexity provided by adult *C. gigas* may create structural refugia which could additionally alter consumption by predators (Grabowski & Powers, 2004; Alexander et al., 2012; Bertolini et al., 2018) and lead to *M. edulis* persistence after invasion (Reise et al. 2017b).

Of course, other life history traits also contribute to invasion success, such as the high growth rates, or the high fecundity of invaders (Troost 2010). However, the results presented here corroborate with field patterns of *C. gigas* invasion (e.g. Reise et al. 2017b), and further inform patterns of invasion success potentially owing to the lack of biotic resistance shown by native predators. In our FR experiments, green crabs consumed similar numbers of *M. edulis* and *C. gigas* at high densities, suggesting they may exert some control over both invasive and native populations. The preference for *M. edulis* over *C. gigas* in the prey switching experiments further informs their interaction, whereby *M. edulis* is disproportionately consumed whilst *C. gigas* is alleviated from predation pressure, inferring a lack of predator-driven biotic resistance towards *C. gigas* invasion. Thus, we advocate the combined use of functional responses and prey switching experiments to understand and predict invader success, impacts on native populations, and strength of biotic resistance from native communities.

Author contributions

P.W.S.J., J.W.E.D., L.T.K., & J.T.A.D. conceived the study. P.W.S.J. and J.W.E.D. performed the experiments. P.W.S.J. and R.N.C. conducted statistical analysis. P.W.S.J. wrote the first draft and all authors contributed to writing the final manuscript and gave approval for publication.

Acknowledgements

P.W.S.J. and R.N.C. are funded by the Department for the Economy, Northern Ireland.
J.W.E.D is funded by Inland Fisheries Ireland. We thank two anonymous reviewers for their
valuable comments which have improved the manuscript significantly.

Conflict of interest

The authors declare that they have no conflict of interest

Data accessibility

Data will be made accessible from the Dryad Digital Repository on acceptance.

References

- Alexander M. E., Dick J. T. A., O'Connor, N., Haddaway N., Farnsworth, K. (2012).
Functional responses of the intertidal amphipod *Echinogammarus marinus*: effects of prey
supply, model selection and habitat complexity. Marine Ecology Progress Series 468:191–
202.
- Allen, J. A. (1988). Frequency-dependent selection by predators. Philosophical Transactions
of the Royal Society B: Biological Sciences, 319, 485–503.
- Angelstam, P., Lindström, E., Widén, P. (1984). Role of predation in short-term population
fluctuations of some birds and mammals in Fennoscandia. Oecologia, 62, 199–208.
- Barrios-O'Neill, D., Kelly, R., Dick, J. T. A., Ricciardi, A., MacIsaac, H. J., Emmerson, M.
C. (2016). On the context-dependent scaling of consumer feeding rates. Ecology Letters, 19,
668–678.
- Bates, D., Mächler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models
Using lme4. Journal of Statistical Software, 67, 1-48.

- 451 Bertolini, C., Montgomery, W. I., O'Connor, N. E. (2018) Habitat with small inter-structural
 452 spaces promotes mussel survival and reef generation. *Marine Biology*, 165:163.
- 453 Bolker BM (2008) *Emdbook: ecological models and data in R*. Princeton University Press,
 454 Princeton, NJ.
- 455 Bovy, H. C., Barrios-O'Neill, D., Emmerson, M. C., Aldridge, D. C., Dick, J. T. A. (2015).
 456 Predicting the predatory impacts of the “demon shrimp” *Dikerogammarus haemobaphes*, on
 457 native and previously introduced species. *Biological Invasions*, 17, 597–607.
- 458 Burlakova, L. E., Karatayev, A. Y., Karatayev, V. A. (2012). Invasive mussels induce
 459 community changes by increasing habitat complexity. *Hydrobiologia*, 685, 121–134.
- 460 Burlakova, L. E., Karatayev, A. Y., Padilla, D. K. (2000). The Impact of *Dreissena*
 461 *polymorpha* (Pallas) Invasion on Unionid Bivalves. *International Review of Hydrobiology*,
 462 85, 529–541.
- 463 Carlsson, N. O. L., Bustamante, H., Strayer, D. L., Pace, M. L. (2011). Biotic resistance on
 464 the increase: native predators structure invasive zebra mussel populations. *Freshwater*
 465 *Biology*, 56, 1630–1637.
- 466 Chesson, J. (1978). Measuring preference in selective predation. *Ecology*, 59, 211–215.
- 467 Chesson, J. (1983). The estimation and analysis of preference and its relationship to foraging
 468 models. *Ecology*, 64, 1297–1304.
- 469 Clarke, B. (1962). Balanced polymorphism and the diversity of a sympatric species. In:
 470 Nichols, D. (ed.), *Taxonomy and Geography* (pp. 47–70) Sympatrics Association, Oxford,
 471 UK.
- 472 Cribari-Neto, F., Zeileis, A. (2010). Beta Regression in R. *Journal of Statistical Software*, 34,
 473 1–24.

- 474 Cuthbert, R. N., Dalu, T., Wasserman, R. J., Dick, J. T. A., Mofu, L., Callaghan, A., Weyl, O.
 475 L. F. (2018a). Intermediate predator naïveté and sex-skewed vulnerability predict the impact
 476 of an invasive higher predator. *Scientific Reports*, 8, 14282.
- 477 Cuthbert, R. N., Dickey, J. W. E., McMorro, C., Laverty, C., Dick, J. T. A. (2018b).
 478 Resistance is futile: lack of predator switching and a preference for native prey predict the
 479 success of an invasive prey species. *Royal Society Open Science*, 5, 180339.
- 480 Dare, P. J. (1982). Notes on the swarming behaviour and population density of *Asterias*
 481 *rubens* L. (Echinodermata: Asteroidea) feeding on the mussel, *Mytilus edulis* L. *ICES Journal*
 482 *of Marine Science*, 40, 112–118.
- 483 Dick, J. T. A., Gallagher, K., Avlijas, S., Clarke, H. C., Lewis, S. E., Leung, S., Minchin, D.,
 484 Caffrey, J., Alexander, M. E., Maguire, C., Harrod, C., Reid, N., Haddaway, N. R.,
 485 Farnsworth, K. D., Penk, M., Ricciardi, A. (2013). Ecological impacts of an invasive predator
 486 explained and predicted by comparative functional responses. *Biological Invasions*, 15, 837-
 487 846.
- 488 Dick, J. T. A., Alexander, M. E., Jeschke, J. M., Ricciardi, A., MacIsaac, H. J., Robinson, T.
 489 B., Kumschick, S., Weyl, O. L. F., Dunn, A. M., Hatcher, M. J., Paterson, R. A., Farnsworth,
 490 K. D., Richardson, D. M. (2014). Advancing impact prediction and hypothesis testing in
 491 invasion ecology using a comparative functional response approach. *Biological Invasions*, 16,
 492 735-753.
- 493 Dick, J. T. A., Laverty, C., Lennon, J. J., Barrios-O'Neill, D., Mensink, P. J., Robert Britton,
 494 J., Médoc, V., Boets, P., Alexander, M. E., Taylor, N. G., Dunn, A. M., Hatcher, M. J.,
 495 Rosewarne, P. J., Crookes, S., MacIsaac, H. J., Xu, M., Ricciardi, A., Wasserman, R. J.,
 496 Ellender, B. R., Weyl, O. L. F., Lucy, F. E., Banks, P. B., Dodd, J. A., MacNeil, C., Penk, M.
 497 R., Aldridge, D. C., Caffrey, J. M. (2017). Invader Relative Impact Potential: a new metric to

- understand and predict the ecological impacts of existing, emerging and future invasive alien species. *Journal of Applied Ecology*, 54, 1259-1267.
- Dickey, J. W. E., Cuthbert, R. N., Rea, M., Lavery, C., Crane, K., South, J., ... Dick, J. T. A. (2018). Assessing the relative potential ecological impacts and invasion risks of emerging and future invasive alien species. *NeoBiota*, 40, 1-24.
- Dolmer, P. (1998). The interactions between bed structure of *Mytilus edulis* L. and the predator *Asterias rubens* L. *Journal of Experimental Marine Biology and Ecology*, 228, 137-150.
- Ebling, F. J., Kitching, J. A., Muntz, L., & Taylor, C. M. (1964). The Ecology of Lough Ine. *Journal of Animal Ecology*, 33, 73-82.
- Edelist, D., Rilov, G., Golani, D., Carlton, J. T., & Spanier, E. (2013). Restructuring the Sea: profound shifts in the world's most invaded marine ecosystem. *Diversity and Distributions*, 19, 69-77.
- Grabowski, J., & Powers, S. (2004). Habitat complexity mitigates trophic transfer on oyster reefs. *Marine Ecology Progress Series*, 277, 291-295.
- Greenwood, J. J. D., & Elton, R. A. (1979). Analysing experiments on frequency-dependent selection by predators. *Journal of Animal Ecology*, 48, 721-737.
- Griffen, B. D., & Delaney, D. G. (2007). Species invasion shift the importance of predator dependence. *Ecology*, 88, 3012-3021.
- Griffiths, C. L., & Seiderer, J. L. (1980). Rock-lobsters and mussels - Limitations and preferences in a predator-prey interaction. *Journal of Experimental Marine Biology and Ecology*, 44, 95-109.
- Griffiths, C. L., & Richardson, C. A. (2006). Chemically induced predator avoidance behaviour in the burrowing bivalve *Macoma balthica*. *Journal of Experimental Marine Biology and Ecology*, 331, 91-98.

- 523 Guy, C., Blight, A., Smyth, D., & Roberts, D. (2018). The world is their oyster: Differences
 524 in epibiota on sympatric populations of native *Ostrea edulis* and non-native *Crassostrea*
 525 *gigas* (*Magallana gigas*) oysters. *Journal of Sea Research*, 140, 52-58.
- 526 Hall, S. J., Todd, C. D., & Gordon, A. D. (1982). The influence of ingestive conditioning on
 527 the species selection in *Aeolidia papillosa* (Mollusca: Nudibranchia). *Journal of Animal*
 528 *Ecology*, 51, 907-921.
- 529 Herbert, R. J. H., Humphreys, J., Davies, C. J., Roberts, C., Fletcher, S., & Crowe, T. P.
 530 (2016). Ecological impacts of non-native Pacific oysters (*Crassostrea gigas*) and
 531 management measures for protected areas in Europe. *Biodiversity and Conservation*, 25,
 532 2835-2865.
- 533 Kochmann, J., Buschbaum, C., Volkenborn, N., & Reise, K. (2008). Shift from native
 534 mussels to alien oysters: Differential effects of ecosystem engineers. *Journal of Experimental*
 535 *Marine Biology and Ecology*, 364, 1-10.
- 536 Lavery, C., Green, K. D., Dick, J. T. A., Barrios-O'Neill, D., Mensink, P. J., Médoc, V., ...
 537 Gallagher, C. (2017). Assessing the ecological impacts of invasive species based on their
 538 functional responses and abundances. *Biological Invasions*, 19, 1653-1665.
- 539 Leonard, G. H., Bertness, M. D., & Yund, P. O. (1999). Crab predation, waterborne cues, and
 540 inducible defences in the blue mussel, *Mytilus edulis*. *Ecology*, 80, 1-14.
- 541 Markert A., Esser, W., Frank, D., Wehrmann, A., Exo, K.M. (2013). Habitat change by the
 542 formation of alien *Crassostrea*-reefs in the Wadden Sea and its role as feeding sites for
 543 waterbirds. *Estuarine, Coastal and Shelf Science*, 131, 41-51.
- 544 Mascaró, M., & Seed, R. (2001). Choice of prey size and species in *Carcinus maenas* (L.)
 545 feeding on four bivalves of contrasting shell morphology. *Hydrobiologia*, 449, 159-170.

- 546 Miron, G., Audet, D., Landry, T., & Moriyasu, M. (2005). Predation potential of the invasive
 547 green crab (*Carcinus maenas*) and other common predators on commercial bivalve species
 548 found on Prince Edward Island. *Journal of Shellfish Research*, 24, 579-586.
- 549 Molnar, J. L., Gamboa, R. L., Revenga, C., & Spalding, M. D. (2008). Assessing the global
 550 threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*,
 551 6, 485-492.
- 552 Murdoch, W. W. (1969). Switching in general predators: Experiments on predator specificity
 553 and stability of prey populations. *Ecological Monographs*, 39, 335-354.
- 554 Murdoch, W. W., & Oaten, A. (1975). Predation and population stability. *Advances in*
 555 *Ecological Research*, 9, 1-131.
- 556 Murray, L. G., Seed, R., & Jones, T. (2007). Predicting the impacts of *Carcinus maenas*
 557 predation on cultivated *Mytilus edulis* beds. *Journal of Shellfish Research*, 26, 1089-1098.
- 558 Nehls, G., Hertzler, I., & Scheiffarth, G. (1997). Stable mussel *Mytilus edulis* beds in the
 559 Wadden Sea - They're just for the birds. *Helgoländer Meeresuntersuchungen*, 51, 361-372.
- 560 Paine, R. T. (1974). Intertidal community structure. *Oecologia*, 15, 93-120.
- 561 Pritchard, D. W., Paterson, R. A., Bovy, H. C., & Barrios-O'Neill, D. (2017). frair : an R
 562 package for fitting and comparing consumer functional responses. *Methods in Ecology and*
 563 *Evolution*, 8, 1528-1534.
- 564 R Development Core Team. (2017). R: A Language and Environment for Statistical
 565 Computing. R Foundation for Statistical Computing, Vienna.
- 566 Raw, J. L., Miranda, N. A. F., & Perissinotto, R. (2013). Chemical Cues Released by an alien
 567 invasive aquatic gastropod drive its invasion success. *PLoS ONE*, 8, e64071.
- 568 Real, L. A. (1977). The kinetics of functional response. *The American Naturalist*, 111, 289-
 569 300.

- 570 Reise, K., Buschbaum, C., Büttger, H., Rick, J., & Wegner, K. M. (2017a). Invasion
571 trajectory of Pacific oysters in the northern Wadden Sea. *Marine Biology*, 164, 68.
- 572 Reise, K., Buschbaum, C., Büttger, H., & Wegner, K. M. (2017b). Invading oysters and
573 native mussels: from hostile takeover to compatible bedfellows. *Ecosphere*, 8, e01949.
- 574 Ruesink, J. (2007). Biotic resistance and facilitation of a non-native oyster on rocky shores.
575 *Marine Ecology Progress Series*, 331, 1-9.
- 576 Saier, B. (2001) Direct and indirect effects of seastars *Asterias rubens* on mussel beds
577 (*Mytilus edulis*) in the Wadden Sea. *Journal of Sea Research*, 46, 29-42.
- 578 Salo, P., Korpimäki, E., Banks, P. B., Nordström, M., & Dickman, C. R. (2007). Alien
579 predators are more dangerous than native predators to prey populations. *Proceedings.*
580 *Biological Sciences*, 274, 1237-1243.
- 581 Sanders, N. J., Gotelli, N. J., Heller, N. E., & Gordon, D. M. (2003). Community disassembly
582 by an invasive species. *Proceedings of the National Academy of Sciences of the United*
583 *States of America*, 100, 2474-2477.
- 584 Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., ...
585 Essl, F. (2017). No saturation in the accumulation of alien species worldwide. *Nature*
586 *Communications*, 8, 14435.
- 587 Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., ...
588 Essl, F. (2018). Global rise in emerging alien species results from increased accessibility of
589 new source pools. *Proceedings of the National Academy of Sciences of the United States of*
590 *America*, 115, E2264-E2273.
- 591 Sih, A., Bolnick, D. I., Luttbeg, B., Orrock, J. L., Peacor, S. D., Pintor, L. M., ... Vonesh, J.
592 R. (2010). Predator-prey naïveté, antipredator behavior, and the ecology of predator
593 invasions. *Oikos*, 119, 610-621.

- 594 Skein, L., Robinson, T. B., & Alexander, M. E. (2018). Impacts of mussel invasions on the
595 prey preference of two native predators. *Behavioral Ecology*, 29, 353-359.
- 596 Sousa, R., Gutiérrez, J. L., & Aldridge, D. C. (2009). Non-indigenous invasive bivalves as
597 ecosystem engineers. *Biological Invasions*, 11, 2367-2385.
- 598 Troost, K. (2010). Causes and effects of a highly successful marine invasion: Case-study of
599 the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries.
600 *Journal of Sea Research*, 64, 145-165.
- 601 van der Zee, E. M., van der Heide, T., Donadi, S., Eklöf, J. S., Eriksson, B. K., Olff, H., ...
602 Piersma, T. (2012). Spatially extended habitat modification by intertidal reef-building
603 bivalves has implications for consumer-resource interactions. *Ecosystems*, 15, 664-673.
- 604 Waser, A. M., Splinter, W., & van der Meer, J. (2015). Indirect effects of invasive species
605 affecting the population structure of an ecosystem engineer. *Ecosphere*, 6, 1-12.
- 606 Weissburg, M. J., & Zimmer-Faust, R. K. (1994). Odor plumes and how blue crabs use them
607 in finding prey. *Journal of Experimental Biology*, 197, 349-375.
- 608 Whiteley, D. A. A., Owen, D. F., & Smith, D. A. S. (1997). Massive polymorphism and
609 natural selection in *Donacilla cornea* (Poli, 1791) (Bivalvia: Mesodesmatidae). *Biological*
610 *Journal of the Linnean Society*, 62, 475-494.

Using functional responses and prey switching to quantify invasion success of the Pacific oyster, *Crassostrea gigas*

Patrick W.S. Joyce, James W.E. Dickey, Ross N. Cuthbert, Jaimie, T.A. Dick, Louise Kregting

- Functional responses and prey switching may predict invader success
- Predation by two predators towards native and invasive bivalve prey was quantified
- Both predators consumed native prey at a higher rate than invasive prey
- Predators disproportionately consumed native prey when prey occurred simultaneously
- Invasion success is mediated through differential levels of biotic resistance