


Relationship between shell integrity of pelagic gastropods and carbonate chemistry parameters at a Scottish Coastal Observatory monitoring site

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Ocean acidification (OA), the anthropogenic carbon dioxide-induced changes in seawater carbonate chemistry, is likely to have a significant impact on calcifying plankton. Most planktonic studies on OA are based on “one-off” cruises focused on offshore areas while observations from inshore waters are scarce. This study presents the first analysis on the shell integrity of pelagic gastropods (holoplanktonic pteropods and planktonic larvae of otherwise benthic species) at the Scottish Coastal Observatory monitoring site at Stonehaven on the east coast of Scotland. The shell integrity of archived pelagic gastropods specimens from 2011 to 2013 was examined using Scanning Electron Microscopy and the relationship with OA (pH and aragonite saturation, Ω_{arg}) and other environmental parameters was investigated. Evidence of shell dissolution was detected in all analysed taxa even though the seawater was supersaturated with respect to aragonite. The shell condition matched the temporal pattern observed in Ω_{arg} with higher proportion of dissolution associated with decreasing Ω_{arg} , suggesting that the seasonality component of carbonate chemistry might affect the shell integrity of pelagic gastropods. The proportion of shell dissolution differed significantly between larvae and adult stages of pteropods, supporting the hypothesis that early-life stages would be more vulnerable to OA-induced changes. Our data also suggest that sensitivity to OA may differ even between closely related taxonomic groups. The strong interannual variability revealed by the year-to-year shell dissolution and Ω_{arg} illustrates the difficulty in assessing the plankton response to OA in the field and the value of time series studies.

Keywords: calcifying plankton, gastropod larvae, ocean acidification, pteropods, Scottish Coastal Observatory, time series.

Introduction

Since the beginning of the industrial period, human activities have resulted in a 40% increase in the concentration of

atmospheric carbon dioxide (CO_2) concentration. This increase is expected to continue throughout the next century (IPCC, 2014; Le Quéré *et al.*, 2018). Global oceans have absorbed about a third

of anthropogenic CO₂ emissions (Khaliwala *et al.*, 2013; Le Quéré *et al.*, 2018). This absorption induces changes in seawater chemistry, including a decline in pH and carbonate ion concentration (Feely *et al.*, 2012). This process, called ocean acidification (OA), poses a significant threat to a wide range of marine organisms, including commercially exploited species (Doney *et al.*, 2009; Mangi *et al.*, 2018). Although significant variation in the sensitivity of different taxa has been described in Kroeker *et al.* (2013), OA is likely to have a major impact on calcifying plankton due to more corrosive conditions under which shell development becomes more energetically costly (Gazeau *et al.*, 2013).

Initially most studies investigating the impacts of OA on calcifying plankton focused on coccolithophores (single-celled phytoplankton) with mixed results (Meyer and Riebesell, 2015) and most field studies showing no relationship between carbonate parameters and coccolithophore calcification (Smith *et al.*, 2012; Marañón *et al.*, 2016; León *et al.*, 2018; among others). In contrast, in comparison to coccolithophores, other planktonic calcifying groups of ecological significance have received less attention (Doney *et al.*, 2009). Pelagic shelled gastropods (hereafter pelagic gastropods) are molluscs including the pelagic larvae of otherwise benthic gastropod species (hereafter meroplanktonic gastropod larvae). Pelagic gastropods also include some holoplanktonic gastropods, such as euthecosomatous pteropods (i.e. with external shell; hereafter pteropods), whose entire life cycle is planktonic (Lalli and Gilmer, 1989). This group is widely distributed in the world's oceans, playing a significant role in the marine environment (Gazeau *et al.*, 2013). They represent an important component of the pelagic food web linking phytoplankton and larger pelagic predators such as carnivorous zooplankton, fish, cephalopods, and birds (Lalli and Gilmer, 1989). Pelagic gastropods also play an important role in biogeochemical cycles of the ocean. Their shells act as ballast to rapidly transport carbon to the ocean bottom (Francois *et al.*, 2002). These shells can be comprised aragonite, sometimes calcite and in several taxa, layers of both calcite and aragonite (Addadi *et al.*, 2006).

An increasing number of recent publications is focusing on the potential impact of OA on pelagic gastropods (Gazeau *et al.*, 2013; Manno *et al.*, 2017). The suborder Euthecosomata, one of the most abundant and important pelagic gastropod groups (Bednaršek *et al.*, 2012a), has been identified as a proxy species for assessing the biological effects of OA (Bednaršek *et al.*, 2017a). Their shell mineralogy (aragonite) makes this group particularly sensitive to changes caused by OA since aragonite is ~50% more soluble in water than calcite (Fabry *et al.*, 2008). Although the response of pteropods to OA includes several physiological and behavioural aspects (Manno *et al.*, 2017), their vulnerability is mostly associated with rapid shell dissolution (Bednaršek *et al.*, 2019). The latter is also an indicator of the organism's metabolic condition in response to OA (Bednaršek *et al.*, 2017a) since the ability of pteropods to repair and maintain their shells requires additional energetic costs (Peck *et al.*, 2018). Our understanding of the potential effects of OA on benthic gastropods in the field is more limited (Gazeau *et al.*, 2013). Studies performed on a diverse range of marine shelled mollusc species suggest that different life stages may have a different tolerance to OA (Gazeau *et al.*, 2013). Although pteropods and many benthic gastropods produce free-swimming larvae as part of their life cycles (Lalli and Gilmer, 1989), the biological impacts of OA on early developmental stages have been considerably less studied in comparison to adult phases (Zhang *et al.*, 2014; Thabet *et al.*, 2015; Gardner

et al., 2018). Available literature suggest pelagic larvae are the most sensitive life history stage to OA, with the majority of studies reporting negative effects on this critical stage (Comeau *et al.*, 2010; Gazeau *et al.*, 2013; Kroeker *et al.*, 2013; Parker *et al.*, 2013; Waldbusser *et al.*, 2015, among others). This enhanced sensitivity of early life history stages in molluscs is not universal across all taxonomic groups (Kroeker *et al.*, 2013). Most OA research on gastropod larvae to date is short-term and laboratory based and extrapolation to natural conditions at sea is not straightforward (Parker *et al.*, 2013). As a result, the biological impacts of OA on gastropod early developmental stages in the field are still not well understood.

Pelagic gastropods distribution patterns show higher abundances in coastal areas (Gazeau *et al.*, 2013) and continental shelves (Bednaršek *et al.*, 2012a). These systems are likely to be more vulnerable to OA impacts than open waters (Duarte *et al.*, 2013) due to a greater temporal (daily and/or seasonal) variability of carbonate parameters (Kitidis *et al.*, 2012). Little research has been conducted on the impact of OA on coastal marine gastropods (Wahl *et al.*, 2016; González-Delgado and Hernández, 2018), in part due to the lack of time-series including concomitant chemical and biological data (Manno *et al.*, 2017). The latter are crucial to determine OA long-term trends and to distinguish between natural variability and anthropogenic forcing (Ostle *et al.*, 2016). In this context, the Scottish Coastal Observatory (SCOs) monitoring site at Stonehaven is providing baseline information about the seasonality and interannual variability of carbonate parameters, along with temperature, salinity, nutrients, and plankton community in inshore waters in the western part of the northern North Sea (Bresnan *et al.*, 2016; León *et al.*, 2018).

This study presents an investigation on the seasonal patterns of abundance, composition, and shell integrity of the pelagic gastropod community in coastal waters of the north western North Sea. Three years of monthly samples of pelagic gastropod species collected at the SCOs monitoring site at Stonehaven (east coast of Scotland) were analysed to investigate shell integrity of the individuals present. The site is characterized by a significant influence of the North Atlantic coastal southward flow and tidal currents, causing a strong mixing of the water column. This hydrography makes Stonehaven representative of mixed coastal systems exposed to offshore waters. To assess the potential impacts of OA on the pelagic gastropod community in Scottish waters, the relationship between temporal variation of shell dissolution with carbonate chemistry and environmental variables at the site was investigated.

Material and methods

Sampling site

Located 5 km offshore from the town of Stonehaven in the North East of Scotland (56°57.8'N 02°06.2'W; Figure 1), the Stonehaven monitoring site is operated by Marine Scotland Science as part of the SCOs. The site has a depth of ~50 m and is situated 5 km offshore from a relatively smooth coastline, making it exposed to storms in the North Sea. Along with the physical parameters temperature, salinity, and Secchi disc depth, seawater samples have been collected weekly (weather permitting) since monitoring began in 1997 to determine chlorophyll, inorganic nutrients, phytoplankton, and zooplankton species composition. Monitoring of carbonate chemistry parameters at the site started in 2009 with the collection of

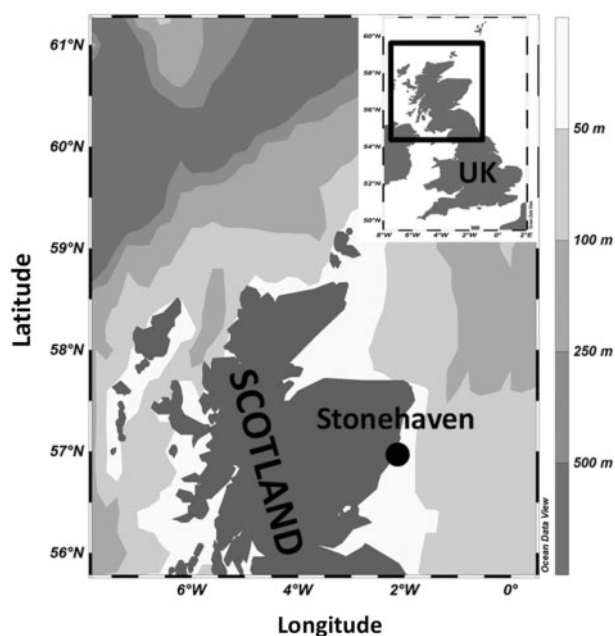


Figure 1. Location of Stonehaven monitoring site (redrawn from León *et al.*, 2018).

additional seawater for the determination of “Total Alkalinity” (TA) and “Dissolved Inorganic Carbon” (DIC). Further information about the Stonehaven monitoring site can be found in Bresnan *et al.* (2015, 2016).

Environmental variables

Water samples for the determination of salinity were taken at 1 and 45 m depth using Niskin bottles fitted with digital thermometers (RTM 4002X, SIS, Germany) to record water temperature. Salinity samples were stored in glass bottles, which were dried and sealed to prevent the formation of salt crystals and evaporation, at temperature-controlled environment until analysis in the laboratory. Salinity analyses were performed using a Guildline Portasal Salinometer Model 8410A. The salinometer was previously standardized using IAPSO standard seawater. A Secchi disc was used to measure the turbidity in the water column.

Carbonate chemistry

Between 2009 and 2013, additional water samples were collected at 1 and 45 m to determine carbonate chemistry parameters and transferred to 250-ml glass bottles. After collection, samples were immediately mixed with 50- μ l saturated HgCl_2 for preservation and stored at room temperature until analysis at the National Oceanography Centre, Southampton. DIC and TA were determined colorimetrically and potentiometrically respectively using a Versatile Instrument for Analysis of Titration Alkalinity (VINDTA 3C, Marianda, Germany), according to the procedures of Dickson *et al.* (2007). Seawater pH and aragonite saturation state (Ω_{arg}) were derived from the variables TA, DIC, temperature, and salinity using the programme CO_2SYS (version 2.1; Pierrot *et al.*, 2006). Ω_{arg} is a measure of the chemical potential of calcifying marine species to form aragonite skeletons or shells in seawater. When Ω_{arg} is < 1.0 , it indicates that aragonite is unstable and biogenic materials made out of calcium carbonate

will dissolve (Doney *et al.*, 2009). Calcite saturation (Ω_{cal}) has not been derived in this study since most pelagic gastropods build their shells from aragonite (Ponder *et al.*, 2008; Bednaršek *et al.*, 2012a). Further information on Ω_{cal} and other carbonate parameters (DIC and TA) at the site during the study period can be found in León *et al.* (2018). The gap in the 2011 data was due to logistical reasons.

Biological sampling

Integrated samples from the upper part of the water column were collected using a 10 m Lund tube for pigment analysis. A volume of 0.5–1 l of seawater, depending on the time of the year, was filtered through GF/F glass fibre filters and stored frozen at -80°C until analysis. Chlorophyll was extracted using buffered acetone for 24 h. Pigment concentration was determined using a Turner AU fluorometer according to the fluorometric method of Arar and Collins (1992). The latter includes an acidification step to “correct” chlorophyll *a* for the presence of phaeopigments. Given that little difference has been found between corrected and uncorrected chlorophyll *a* (Smith *et al.*, 2007), uncorrected data have been used in this study. Zooplankton samples were collected using a Bongo plankton net (0.40 m diameter) fitted with a 68 and 200 μm mesh. The nets were hauled twice vertically from near bottom (45 m) to surface at a speed of 2–3 m s^{-1} to get two set of samples. Each set was immediately preserved in 4% borax buffered formaldehyde and 95% ethanol respectively for later analysis in the laboratory. Zooplankton from the 200- μm mesh cod-end were counted and identified under a Zeiss Stemi-11 stereomicroscope for determination of total abundance and species composition while the remaining samples were stored for future use. Data collected between 1999 and 2013 are used to describe the seasonality of this zooplankton group.

Shell dissolution

The integrity of the pelagic gastropod shells was assessed by identifying evidences of shell dissolution using Scanning Electron Microscopy (SEM). Specimens for SEM analysis were isolated from archived zooplankton samples collected at Stonehaven. Note that only samples preserved in ethanol (Oakes *et al.*, 2019) were used in this study. Prior to SEM analysis, shells were treated to obtain clean surfaces and remove the outer organic layer (periostracum) following the method described by Bednaršek *et al.* (2012b, 2016). The shells were carefully mounted on aluminium SEM stubs using fine brushes and sputter-coated with gold/palladium under low vacuum conditions using a Quorum Q150T ES. The specimens were examined using a Zeiss EVO MA10 SEM at the Institute of Medical Sciences (University of Aberdeen) by moving in small incremental steps around the shell. Only individuals with non-mechanical shell damage were considered for further analysis. SEM micrographs were used to identify specimens and to determine if signs of dissolution on the outer shell surface were present. One monthly sample between 2011 and 2013 was analysed in this study.

Typically, the pteropod genus *Limacina* (suborder Euthecosomata) has a shell microstructure consisting of a thick underlying crossed-lamellar layer and an upper thin prismatic layer (Bé and Gilmer, 1977). Most larval shells of marine gastropods are characterized by an outer homogeneous and an inner aragonitic crossed-lamellar microstructure (Carter and Clark, 1985; Bandel, 1990). Based on this information, we have assumed

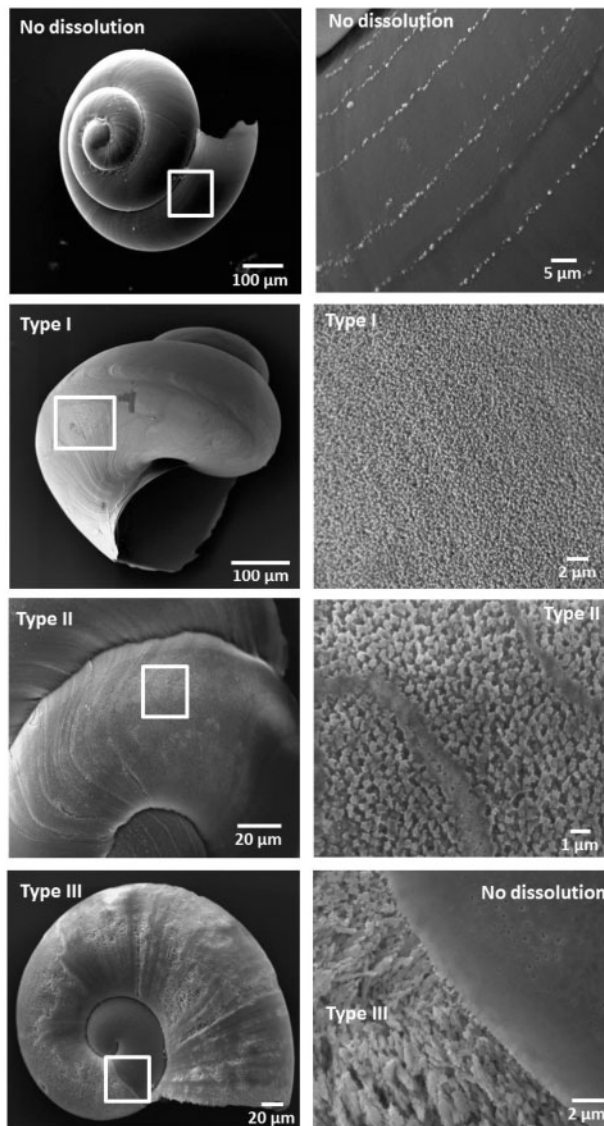


Figure 2. SEM micrographs of pelagic gastropod shells showing different types of dissolution according to categories described by Bednaršek et al. (2012b).

a similar shell microstructure for both pelagic gastropod groups: pteropods and meroplanktonic gastropod larvae. If any dissolution was present, specimens were categorized into three levels of dissolution based on the degree of shell damage using the criteria defined in Bednaršek et al. (2012b) (Figure 2): Type I, partial dissolution of the outer layer; Type II, partial disappearance of the outer layer and exposure of the crossed-lamellar layer; Type III, signs of dissolution of the crossed-lamellar layer. For simplicity, given that the last two types indicate severe damage on shell integrity (Bednaršek et al., 2012b), we will refer to type I and types II/III as mild and severe damage, respectively.

Statistical analysis

The relationships between pelagic gastropod abundance and shell dissolution with carbonate chemistry and environmental data was examined using several tests. For each month, mean values of temperature, salinity, pH, and Ω_{arg} were calculated from samples

collected at 1 and 45 m to be compared to the pelagic gastropod data. Pearson's correlation analyses between some variables were performed. A one-way ANOVA was used to test for significant differences ($\alpha = 0.05$) in abundance and occurrence of shell dissolution among pelagic gastropods groups. Principal Component Analyses (PCAs) were performed to assess seasonal patterns of pelagic gastropod abundance and shell dissolution incidence, as well as to explore the influence of physicochemical variables. The first PCA was conducted on meroplanktonic gastropod larvae/pteropods abundance while a second analysis was conducted on total pelagic gastropod shell dissolution. The other input variables for the PCAs were: temperature, salinity, chlorophyll, water transparency, pH, and Ω_{arg} . Statistical tests were conducted using the software package Statistica 7.1 (Statsoft, Inc., 1984–2005).

Results

Environmental variables

Monthly averages of environmental parameters at Stonehaven between 1997 and 2013 are shown in Figure 3. Winter months were characterized by colder and less saline waters, with minimum temperatures and salinity (up to 4.37°C and 33.33, respectively) usually recorded in February/March (Figure 3a and b). Both seawater temperature and salinity increased from March/April until late summer (August/September), reaching maximum absolute values in the time-series of 16.23°C and 34.99, respectively. Chlorophyll concentrations were low in winter, increased in spring months and peaked in May/June (Figure 3c), declining over summer months when seawater temperature and salinity increased. Considerable intra-month and interannual variation of environmental variables, particularly in salinity and chlorophyll, was also observed at the site associated mainly with winter–summer and spring–summer periods, respectively (Bresnan et al., 2015, 2016).

Carbonate chemistry

The weekly distribution of carbonate parameters at Stonehaven during the study period is described in León et al. (2018). Here, we include a short description of Ω_{arg} and pH. The temporal distribution of pH showed a clear seasonal pattern (Figure 4a), with values ranging from 7.88 to 8.18. pH was generally lower during winter (December–February), increasing through spring, reaching higher values during summer (June–July) and decreasing throughout autumn. However, minimum and maximum values corresponded to samples collected in summer (June 2013) and spring (April 2012), respectively. Weekly Ω_{arg} distribution (Figure 4b) showed a similar seasonality. Saturation states were generally higher in late spring–summer months (up to 2.7 in May 2012), decreasing during autumn until reaching lower values in winter. Ω_{arg} values exceeded 1 in all samples, indicating that seawater at Stonehaven was supersaturated with respect to aragonite during the study period. The pH and Ω_{arg} data highlight the interannual variability associated with carbonate chemistry at Stonehaven. For instance, pH was particularly variable during autumn periods and Ω_{arg} minima (~ 1.3) were not recorded in winter but during spring (2013). Both pH and Ω_{arg} followed similar patterns at surface and bottom sampling depths.

Pelagic gastropod community composition and abundance

Two species of pteropods (*Limacina retroversa* and *Limacina helicina*) and several meroplanktonic gastropod larvae taxa were

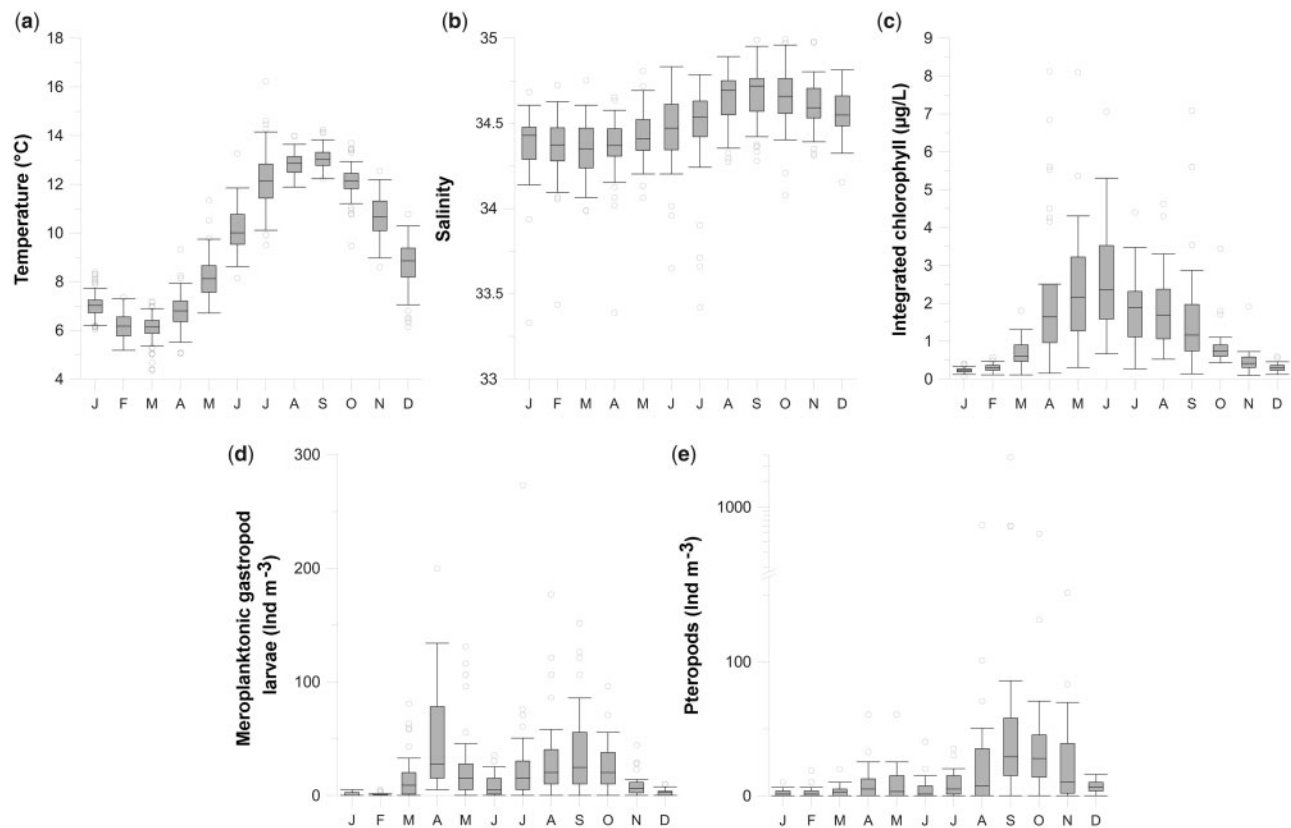


Figure 3. Box whisker plot of (a) temperature, (b) salinity, (c) chlorophyll, (d) meroplanktonic gastropod larvae, and (e) pteropod abundance at Stonehaven for the period 1999–2013. The horizontal line within the box indicates the median value of the weekly datasets recorded in that calendar month, from all years observed. The whiskers represent the maximum and minimum values recorded that calendar month, if within the first quartile. Blank circles show weekly data exceeding these values.

identified within the samples (Figure 5), although only some groups have been identified in this study: *Caecum* spp., *Janthina* spp., family Naticidae, and *Rissoa* spp. Note that *Janthina* spp. (commonly known as bubble rafting snail) is a neustonic taxon (i.e. inhabit the region on or just below the surface) while the remaining taxa are meroplanktonic snails (i.e. benthic species with only some of their lifecycle as part of the plankton, usually larvae stages). Specimens belonging to other taxa (e.g. family Pyramidellidae, order Nudibranchia) were observed in very low numbers and were not considered in the shell integrity analysis.

The distribution of monthly average abundance of pelagic gastropods collected from Stonehaven between 1999 and 2013 shows a clear seasonal trend (Figure 3d and e). Meroplanktonic gastropod larvae peaked in abundance during springtime (April), and late summer (August–September; Figure 3d). Pteropod abundance was low during spring and began to increase through summer months reaching a peak in autumn (mainly September; Figure 3e). The intensity of the peaks in abundance was highly variable between years ranging up to 273 and 9529 individuals per m^{-3} for meroplanktonic gastropod larvae and pteropods, respectively. The temporal distribution of abundance between 2011 and 2013 (Figure 4c and d) is consistent with the general pattern described above. Higher abundances of meroplanktonic gastropod larvae (up to 134 Ind m^{-3}) were observed in spring and late summer while pteropods only peaked in late summer/early autumn (with a maximum of 9529 Ind m^{-3}). The year-to-year data also showed a high interannual variability with marked

lower peaks observed in 2011, particularly in pteropods. Meroplanktonic gastropod larvae and pteropods showed similar abundances ($p > 0.05$) during the study period.

Evidence of shell dissolution

Seventy-three individuals (~16%) of the 450 isolated specimens showed mechanical damage on shells (e.g. from handling while isolated and mounted on SEM stubs) and were not considered further for shell integrity analysis. SEM data showed evidence of shell dissolution in approximately one-third of all the intact (non-mechanical damage) shells examined (125 of the 377 studied). Although the number of damaged (dissolution) shells was significantly higher ($p < 0.05$) in meroplanktonic gastropod larvae (65 specimens; *Caecum* spp., *Janthina* spp., Family Naticidae, and *Rissoa* spp.) than in pteropods (25 specimens), the proportion of shell dissolution was statistically similar in both groups ($p > 0.05$; Figure 6a). The comparison of meroplanktonic gastropod larvae showed a different incidence of shell damage among analysed taxa. With only 10 shells of the 117 individuals examined (~8.5%), the proportion of severe shell dissolution in *Rissoa* spp. specimens was significantly lower ($p < 0.05$) than in *Caecum* spp. individuals, which showed severe damage in almost 70% of shells analysed (25 individuals; Figure 6b). Most evidence of shell dissolution observed in pteropods (~86%) was found in larval individuals, which showed higher ($p < 0.05$) proportion of severe damage compared to adult shells (Figure 6c). The latter only

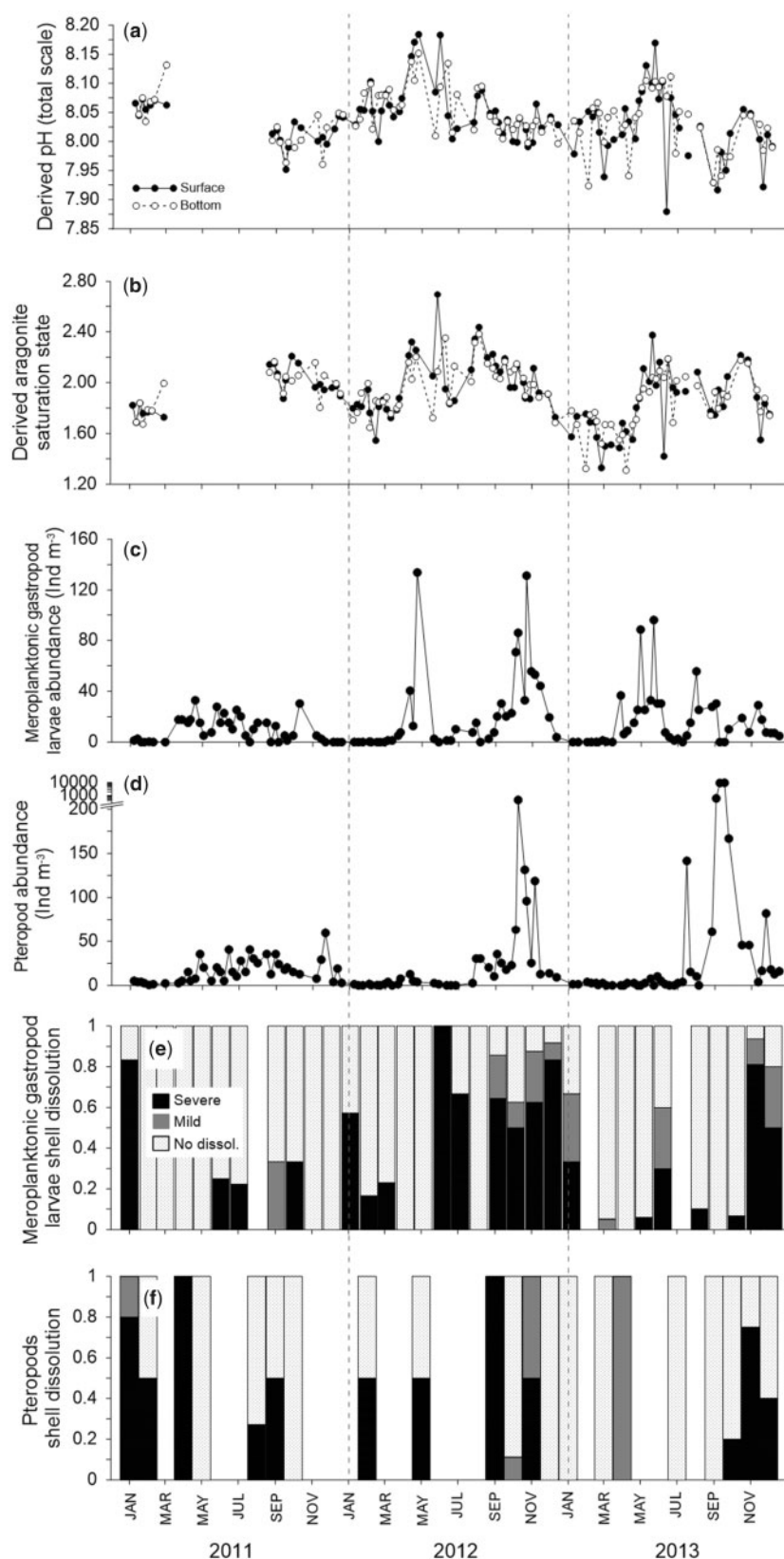


Figure 4. Weekly distribution of (a) pH, (b) aragonite at 1 m (surface; filled circles), and 45 m (bottom; blank circles), (c) meroplanktonic gastropod larvae and (d) pteropods abundance, and monthly distribution of proportion of shell dissolution (bars) grouped by (e) meroplanktonic gastropod larvae and (f) pteropods. Plot (a) (pH) redrawn from León *et al.* (2018).

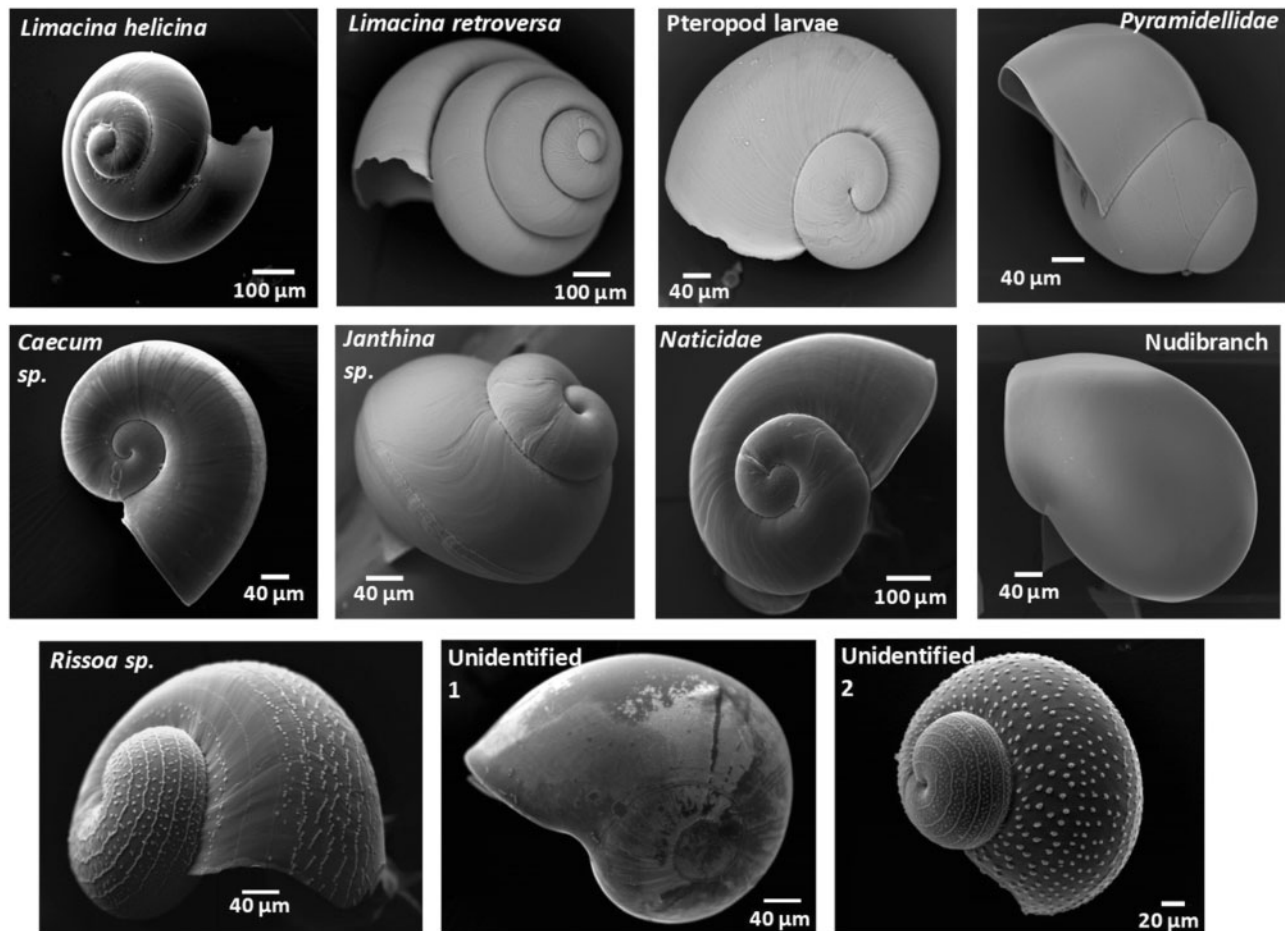


Figure 5. SEM micrographs of pelagic gastropod taxa observed at Stonehaven.

showed evidence of shell dissolution in 4 specimens of the 28 individuals examined, collected in August 2011 (*L. retroversa*; severe), November 2012 (*L. helicina*; mild), and December 2013 (*L. retroversa*; severe).

The analysis of the monthly distribution of shell dissolution revealed seasonality effects over the study period, particularly on meroplanktonic gastropod larvae. Higher proportions of severe damage, ranging between 50% and 100% of specimens examined per sample, were observed in autumn–winter months (Figure 4e), matching both low larvae densities (January 2011–2013, autumn 2013) and peaks in abundance (autumn 2012) (Figure 4c). No evidence or only a low proportion of severe damage on meroplanktonic gastropod larvae were observed mostly in spring, coinciding with their abundance peaks, and summer months (Figure 4c and e), although high proportions of shell dissolution were also observed in summer 2012/2013. Our data also showed a considerable interannual variability, with significant ($p < 0.05$) higher proportions of severe shell damage observed in 2012. Similarly, although a seasonal pattern was less clear, most shell dissolution in pteropods was observed in autumn–winter months. A low proportion of shell damage in this group was observed coinciding with their peaks of abundance in October 2012 and September 2013 (Figure 4d and f). On the contrary, 100% of the specimens in samples collected during some periods of low pteropod abundance (April 2011 and September 2012) showed severe shell damage. Marked differences were detected when comparing the

temporal distribution of shell dissolution among meroplanktonic gastropod larvae groups (Figure 7). Higher proportions of severe damage were observed in autumn–winter months in 2012 and 2013, while specimens belonging to the Family Naticidae showed higher proportions in summer 2012 and December 2013. Severe damage on *Rissoa* spp. shells were observed mostly in individuals collected in 2012, mainly in January and autumn but also in summer months.

Relationships between environmental variables and pelagic gastropods

The PCA examining environmental variables and pelagic gastropod abundance is shown in Figure 8a and b. The first three components were found to be significant, accounting for ~69% of total variation within the data. The first principal component (PC1), representing 29.7% of the variance, was contributed positively by all variables except pH, which was negatively correlated with PC1. Temperature and salinity were the main variables contributing to PC1 with correlation factors of 0.94 and 0.77, respectively, while chlorophyll contribution to PC1 was very low (~0.1). Most of the samples collected in winter–spring showed negative scores for this component (Figure 9a and b), reflecting the influence of seasonal patterns of temperature and salinity on PC1. The second principal component (PC2) explained ~24% of

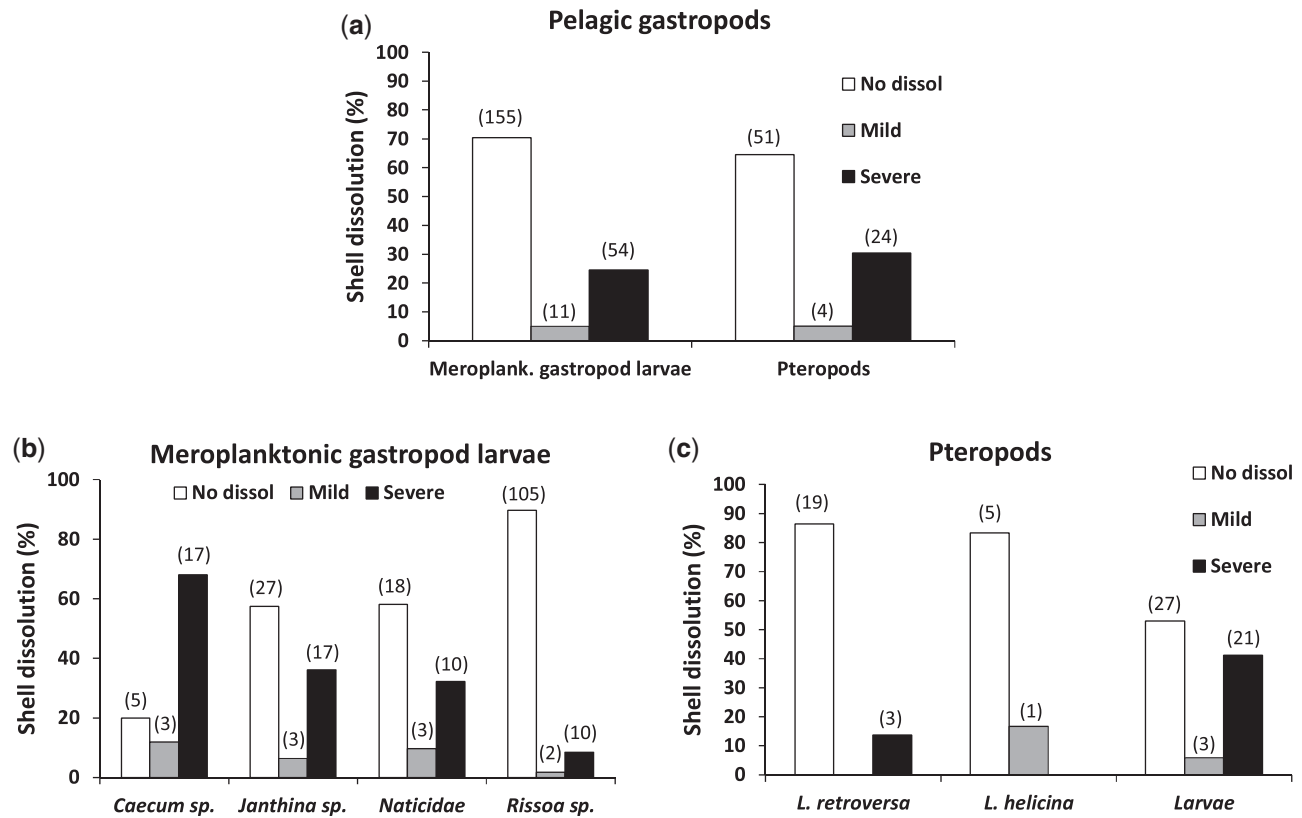


Figure 6. Percentage of shell dissolution in (a) pelagic gastropods, (b) meroplanktonic gastropod larvae, and (c) pteropods. Types II and III are plotted together since both indicate severe damage on shell integrity.

the variability and was mainly contributed (positively) by pH, Ω_{arg} and chlorophyll. The latter variables were positively correlated ($p < 0.05$), with high chlorophyll concentrations matching increased Ω_{arg} . PC2 clearly discriminated most of the samples collected in spring and winter, with positive and negative scores, respectively. Thus, PC2 shows the influence of chlorophyll and carbonate parameters seasonality on data. It is worth noting the low correlations observed between the pelagic gastropod groups and PC1 (~ 0.3), although salinity and pteropods abundance were positively correlated ($p < 0.05$), and particularly between meroplanktonic gastropod larvae and PC2 (~ 0.02). The third component (PC3), representing 15% of the variability, was mainly contributed by the pelagic gastropod groups. Despite the correlation with chlorophyll, PC3 did not explain any seasonal pattern on data. The second PCA, performed with the proportion of severe shell dissolution (Figure 8c and d), showed similar results and accounted for a slightly higher percentage of the variability (73%). The first two components, explaining 57% (31% and 26%, respectively) of the variance, showed the same seasonality as in the first PCA (Figure 9c) and a low correlation with shell dissolution (< 0.1). The latter was the main variable contributing to PC3, which represented 16% of the variance and discriminated most of the samples collected in autumn–winter with positive scores (Figure 9d).

Discussion

Environmental framework

Environmental variables at Stonehaven follow the typical seasonal pattern observed in temperate latitudes. The increase in seawater

temperature and daylight during spring is critical for the initiation of the phytoplankton bloom at this period. Although chlorophyll concentration declines over summer, a less intense phytoplankton bloom is frequently observed in late summer coinciding with temperature and salinity peaks (Bresnan et al., 2015, 2016). The seasonal cycle of salinity at the site is mainly driven by variations of the inflow of Atlantic Water, with saltier values observed in autumn and early winter associated with high Atlantic inflow (Dye et al., 2013), while local freshwater inputs have a sporadic influence. Carbonate chemistry at the site is strongly influenced by phytoplankton patterns (León et al., 2018). Carbonate parameters (except DIC, which follows an opposite pattern) increase with rising phytoplankton biomass and the nutrient uptake associated with intense photosynthesis in spring/summer months and decrease in autumn when respiratory processes by zooplankton are predominant (Kitidis et al., 2012). The variation of pH and Ω_{arg} during the study period is consistent with that pattern and reflects the strong intra-month variability associated with the environmental variables (León et al., 2018). This is particularly clear along the phytoplankton growing period (spring–summer) and during sporadic surface freshwater inputs, with pH fluctuations ~ 0.12 units larger than the average decrease over the study period (~ 0.18). The annual cycle of Ω_{arg} at the site is dominated by supersaturation although the seasonal variations were high (up to twofold). In contrast to coastal seasonally stratified systems (Kitidis et al., 2012), carbonate chemistry seasonality at Stonehaven is consistent with depth due to the typical weak stratification observed at the site (Bresnan et al., 2015, 2016).

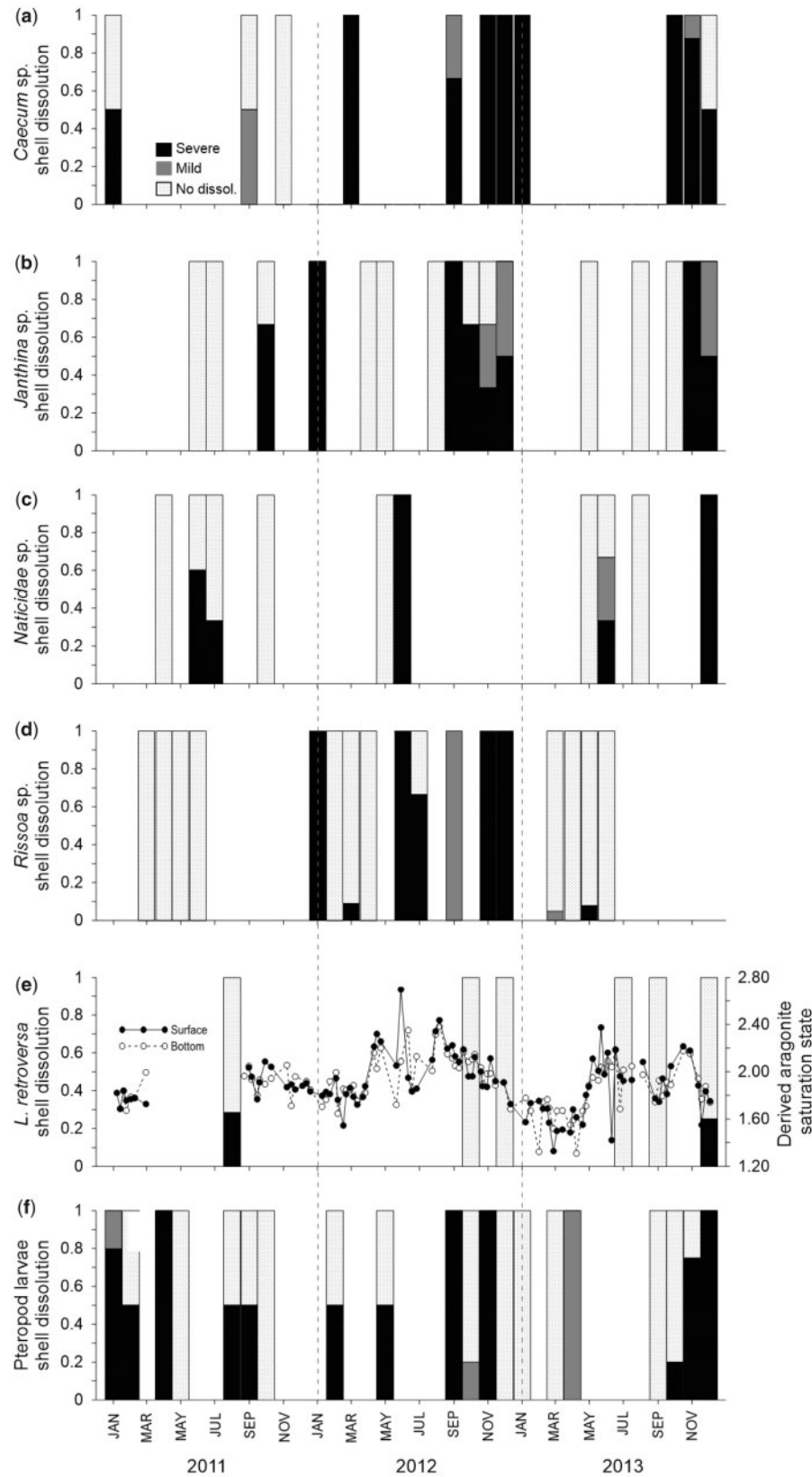


Figure 7. Monthly distribution of proportion of shell dissolution (bars) in (a) *Caecum* spp., (b) *Janthina* spp., (c) *Naticidae*, (d) *Rissoa* spp., (e) *L. retroversa*, and (f) pteropod larvae. Weekly distribution of aragonite at 1 m (surface; filled circles) and 45 m (bottom; blank circles) is also shown (e) to facilitate the comparison between seasonal patterns of shell damage and aragonite saturation states.

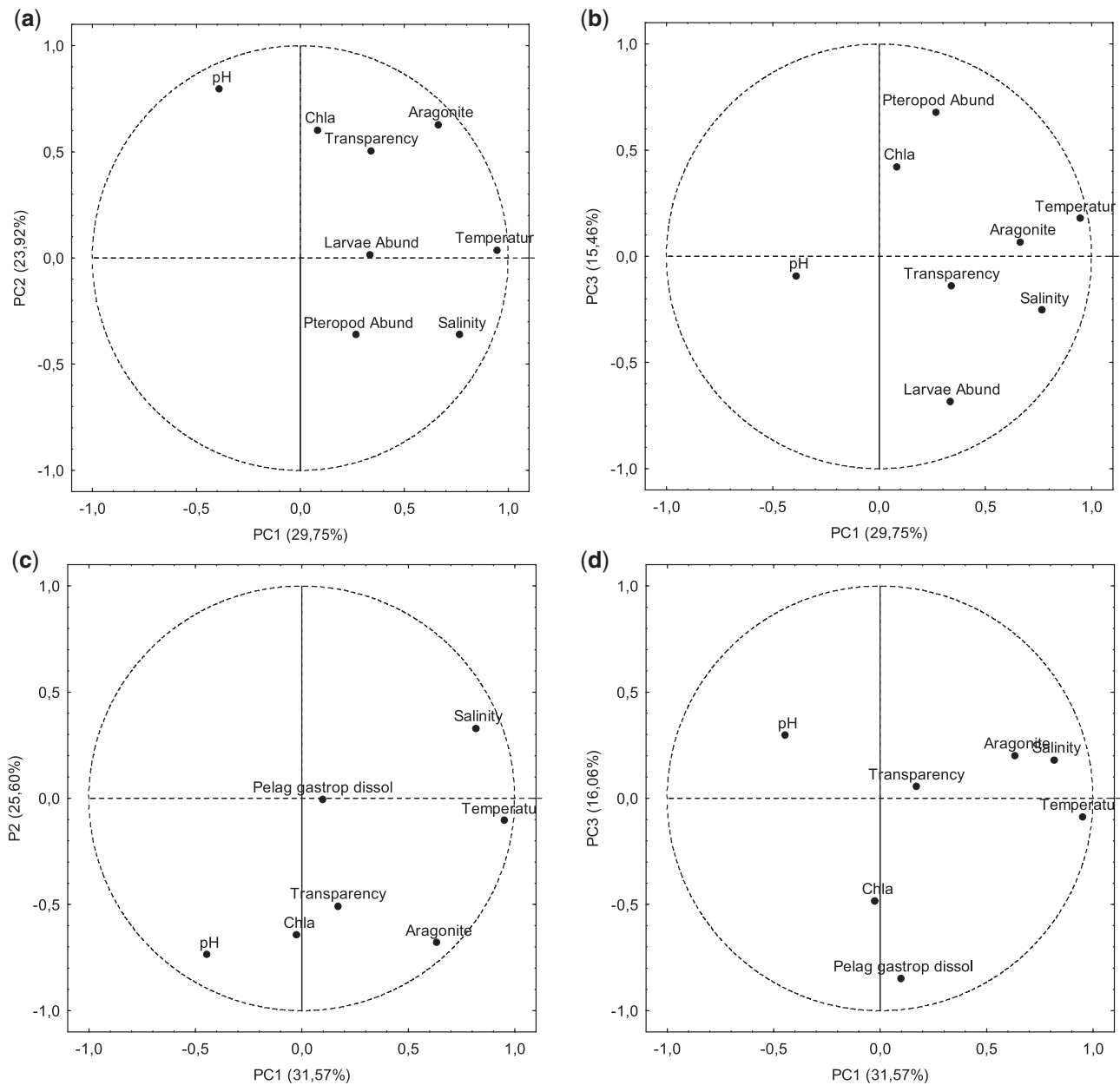


Figure 8. Structure of first three factors extracted from factorial analysis performed for (a, b) abundance of meroplanktonic gastropod larvae/pteropods, and (c, d) pelagic gastropods severe shell dissolution.

Pelagic gastropod community

Seasonality is the main driver of pelagic gastropod abundance at Stonehaven, with peaks matching the initiation of the phytoplankton spring bloom and a less intense second bloom under warmer waters in late summer (Bresnan *et al.*, 2015, 2016). This cycle is consistent with the feeding biology described for both groups, as meroplanktonic gastropod larvae and thecosome pteropods are considered predominantly herbivorous (Lalli and Gilmer, 1989; Hunt *et al.*, 2008; Ponder *et al.*, 2008). The temporal variation of pelagic gastropod abundance during the study period was consistent with that pattern, with the PCA showing a lack of relationship with most of analysed environmental variables but chlorophyll concentration. Our data also showed a high year-to-year variability in pelagic gastropod abundance.

Inter-annual variation is a common phenomenon among grazer pelagic gastropods (Lalli and Gilmer, 1989), which are strongly influenced by inter-annual variability in primary production (Hunt *et al.*, 2008). Our data are consistent with that pattern. Phytoplankton biomass conditions prevalent at Stonehaven during 2012 and 2013 were similar and differed from those in 2011. The highest pelagic gastropod peaks were recorded in 2012–2013 under well-defined phytoplankton blooms. In contrast, significant lower abundances were observed in 2011 coinciding with a strong weekly variability of chlorophyll in spring and the absence of a phytoplankton bloom in late summer (León *et al.*, 2018). However, the low correlation coefficient (-0.43) with chlorophyll suggests that other environmental factors also affect pelagic gastropod abundance at the site. The positive correlation between

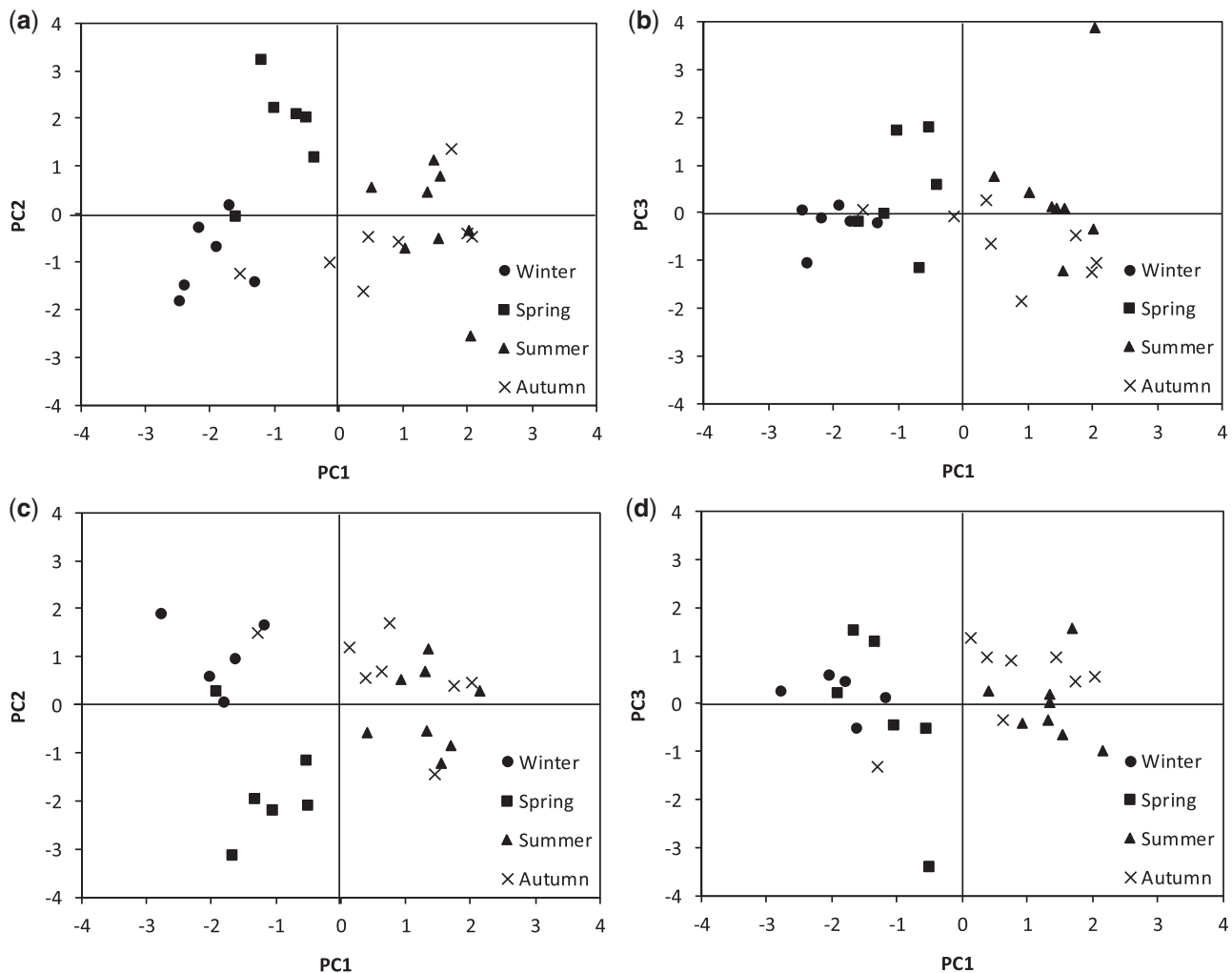


Figure 9. Bi-plot of the scores for the first three factors of each sample used in the factorial analysis performed for meroplanktonic gastropod larvae/pteropods (a, b) abundance and pelagic gastropods (c, d) severe shell dissolution. Scores were grouped seasonally according to the period of the year in which each sample was collected: winter (January–March), spring (April–June), summer (July–September), and autumn (October–December).

salinity and pteropod abundance suggests the advection of Atlantic waters as the origin of pteropod peaks at the site. Pteropods would be advected during summer when the Atlantic inflow increases and peak in September/October when the inflow is maximum, then declining in winter associated with the decreased inflow. Similarly, the lack of correlation with salinity seems to indicate a local origin of meroplanktonic gastropod larvae. The absence of relationship with the other variables suggest that wider environmental factors not analysed in this work (e.g. reproductive pattern, species lifecycle, specific food requirements, and predation; [Absher et al., 2003](#)) may play an important role at the site.

Two shelled pteropod species, *L. helicina* and *L. retroversa*, and the planktonic larvae of several benthic gastropod taxa were found in the Stonehaven samples. Many marine benthic gastropod species produce free-swimming larvae ([Lalli and Gilmer, 1989](#)). However, the identification of meroplanktonic gastropod larvae to species level using morphological criteria is difficult due to the lack of taxonomic literature and expertise on early life stages. This “knowledge gap” hinders the ability to

properly assess the impacts of OA on this group as the structure and calcite/aragonite content of shells may vary among taxa, hence their sensitivity to OA ([Parker et al., 2013](#)). Accordingly, our analysis on shell integrity focused only on those taxa occurring at Stonehaven that were able to be identified with confidence and of which shell composition and microstructure are known: *Caecum* spp., *Janthina* spp., family Naticidae, and *Rissoa* spp. All these taxa are included in the superorder Caenogastropoda ([Ponder et al., 2008](#)), in which shell microstructure is predominantly aragonitic crossed-lamellar, while calcite is rare ([Ponder et al., 2008](#)). Larval shell calcification in molluscs is generally aragonitic, even in species with largely calcitic adult shells ([Carter and Clark, 1985](#)). The latter are typically comprised in similar, if not identical, ultrastructures ([Weiss et al., 2002](#), and references therein). Considering that all the specimens analysed in this study (apart from adult pteropods) corresponded to early developmental stages, our assumptions on shell structure and composition (see Material and methods section) are consistent with the information described above.

Shell dissolution and carbonate chemistry

Pelagic gastropods and particularly pteropods have been proposed as sentinel species for anthropogenic OA change (Bednaršek *et al.*, 2017a). Dissolution of *Limacina* sp. specimens has already been used as an indicator of OA at several marine systems, including the eastern Pacific coast and polar waters (Manno *et al.*, 2007; Bednaršek *et al.*, 2014; Peck *et al.*, 2018). This study demonstrates evidence of shell dissolution on pteropods in the western northern North Sea. In contrast to previous observations, mostly derived from sporadic cruise samplings, our data showed dissolution over the 3-year study period. Shell dissolution was detected mainly on larval stages. Reduced shell weights and increased dissolution have also been reported in adult specimens of several benthic gastropod species under OA conditions in the field (Hall-Spencer *et al.*, 2008; Marshall *et al.*, 2008; Duquette *et al.*, 2017). Our study extends the analysis on shell integrity to the larvae stages showing, to our knowledge, the first field evidence of shell dissolution in early-life stages of benthic gastropods.

Carbonate chemistry seasonality and shell integrity

Little is known about the role of carbonate chemistry seasonality on pelagic gastropods shell integrity. According to the general assumption that calcification and carbonate concentrations are positively correlated (Doney *et al.*, 2009), it could be speculated that the temporal distribution of shell dissolution would reflect seasonal patterns in carbonate parameters, making shell dissolution a useful indicator for shorter OA exposure as well. Our results seem to support this hypothesis. Most of the shell dissolution was observed in autumn–winter months coinciding with the seasonal decline of carbonate parameters, mainly Ω_{arg} . On the contrary, no evidence of shell dissolution or lower proportions of shell damage were observed in spring when Ω_{arg} increased. Furthermore, high proportions of shell damage observed in summer months also matched marked declines in Ω_{arg} . It is worth noting that pteropod shell dissolution is usually associated with exposure to aragonite undersaturated ($\Omega_{\text{arg}} < 1$) and near-saturated ($\Omega_{\text{arg}} \sim 1$) waters (Lischka *et al.*, 2011; Bednaršek *et al.*, 2012b, 2014; Manno *et al.*, 2012; Busch *et al.*, 2014; among others) while pH effects are not so evident. Accordingly, Ω_{arg} has been proposed as a better chemical measure of OA stress than pH (Bednaršek *et al.*, 2019). Carbonate undersaturation events have already been reported in several ocean regions (Bednaršek *et al.*, 2012a, 2014) and are predicted to spread in mid and high latitudes by the middle of this century (Orr *et al.*, 2005). To date, this is not the case observed at Stonehaven where seawater was supersaturated ($\Omega_{\text{arg}} > 1$) during the study period.

Shell dissolution under aragonite supersaturation conditions

Although aragonite was not a limiting factor for calcification at Stonehaven during the study period, almost one-third of the shells examined presented severe damage. Shell dissolution under supersaturated conditions has also been described in Australian tropical waters (Roger *et al.*, 2012), California Current System (Bednaršek and Ohman, 2015), and Greenland Sea (Peck *et al.*, 2018). These observations contradict the chemical definition that calcification is predominant when $\Omega_{\text{arg}} > 1$, while under $\Omega_{\text{arg}} < 1$ biogenic aragonitic dissolution is favoured, illustrating that the real situation in the marine environment is much more complex. Although taxon-specific thresholds of Ω_{arg} have still to be

identified with certainty, $\Omega_{\text{arg}} > 1$ have been suggested as critical values for several calcifying organisms such as shellfish and corals (Atkinson and Cuet, 2008; Waldbusser *et al.*, 2015). A recent meta-analysis study on pteropods set thresholds for mild and severe dissolution at $\Omega_{\text{arg}} = 1.50$ for 5 days and $\Omega_{\text{arg}} = 1.20$ for 14 days, respectively (Bednaršek *et al.*, 2019). Our Ω_{arg} minima fit within that range and are similar to described field thresholds (Bednaršek *et al.*, 2014; Bednaršek and Ohman, 2015). However, in our study those values were only observed sporadically and did not correspond to severe shell dissolution, which was observed mostly under $\Omega_{\text{arg}} > 1.6$. Bacterial metabolic activity can cause corrosive conditions in microenvironments even under $\Omega_{\text{arg}} > 1.0$ (Andersson *et al.*, 2011). Although this factor has not been analysed in this work, it does not seem very feasible considering the number of shells affected by dissolution. Food-depletion can also influence the potential impact of OA on marine calcifiers (Gazeau *et al.*, 2013; Ramajo *et al.*, 2016) by affecting the organisms metabolic state and hence their resilience mechanisms (e.g. shell repair systems; Peck *et al.*, 2018). Contrarily, the PCA did not show any particular relationship between shell damage and chlorophyll seasonality during the study period, in agreement with observations by Bednaršek *et al.* (2017b). Prolonged storage of archived samples in ethanol may also produce dissolution effects (Howes *et al.*, 2017; Oakes *et al.*, 2019). The low damage (only early signs of mild dissolution) and the storage period (~ 100 years) reported by Howes *et al.* (2017) compared to the storage time before our analysis (3–5 years), suggests a negligible influence (if any) of this factor on shell integrity in this study. In fact, for most of analysed taxa, the proportion of shell damage was higher in samples collected in 2012–2013 than in those preserved since 2011.

Several studies highlight exposure history, this is Ω_{arg} conditions experienced by the shells prior to sampling, as the most likely explanation to observed shell dissolution under $\Omega_{\text{arg}} > 1$ (Bednaršek and Ohman, 2015; Peck *et al.*, 2018). Considering Stonehaven hydrography, characterized by a coastal southward flow, and that short-term (i.e. days) exposure is enough to cause shell damage (Bednaršek *et al.*, 2014; Gardner *et al.*, 2018), observed dissolution could be a consequence of exposure to less saturated waters experienced in areas prior to reaching Stonehaven. The potential role of the Atlantic inflow in pteropods life history at the site seems to support this hypothesis. Reduced abundances associated with the decreased inflow in winter would imply a shorter exposure of pteropods to the seasonal decrease in Ω_{arg} . This might explain the lower proportions of shell damage observed in those months compared to meroplanktonic gastropod larvae. Unfortunately, our dataset does not allow this hypothesis to be confirmed. A comprehensive evaluation of the exposure history of the specimens requires using particle tracking models that can integrate both the intensity and the duration of exposure (Manno *et al.*, 2017). This tool has been demonstrated to improve significantly the predictions of pteropod survival scope to $\Omega_{\text{arg}} < 1$ (Bednaršek *et al.*, 2017b). The evidence of shell dissolution and life history of meroplanktonic gastropod larvae at Stonehaven suggest that the seasonality component of Ω_{arg} might imply stressful conditions for pelagic gastropods in autumn–winter that could potentially affect their shell integrity, even under $\Omega_{\text{arg}} > 1$. Similarly, sporadic declines in Ω_{arg} associated with the strong variability of the carbonate chemistry in coastal systems might explain deviations from that seasonal pattern (e.g. summer 2012).

Shell dissolution and life history stages

It has been hypothesized that gastropod early-life history stages will be more sensitive to OA than juveniles and adults, due to the lack of specialized resilience mechanisms and a more soluble shell composition (Parker *et al.*, 2013; Duquette *et al.*, 2017). This hypothesis is supported by experimental observations on pteropods showing a pronounced shell sensitivity of larvae and juveniles individuals to aragonite undersaturation (Comeau *et al.*, 2010; Lischka *et al.*, 2011; Gardner *et al.*, 2018, among others). Although reflecting a sensitivity across all life stages, our data showed a higher proportion of severe shell damage on larval pteropod stages compared to adult specimens. This is, to our knowledge, the first field evidence of a higher sensitivity of pteropod early-life stages to shell dissolution. As such, our results hint at the early-stage stress followed by increased mortality. If the larval stages experience extensive amount of dissolution they are more prone to mortality (Green *et al.*, 2004, 2009) and as such, shell dissolution can shape seasonal population dynamics. Meroplanktonic gastropod larvae also showed evidences of shell dissolution in all analysed taxa, although with significant differences among taxonomic groups. Variation in the sensitivity of larvae of molluscs to OA is common even among closely related species. Genetic variability among taxa may influence their resilience capability (Williamson and Widdicombe, 2018) by affecting shell mineralogical plasticity, mechanical properties, and efficiency of repair systems (Parker *et al.*, 2013). For instance, the presence of amorphous calcium carbonate (more susceptible to dissolution) before the transition to aragonite in early larval stages, may explain the higher susceptibility of protoconches to shell dissolution in some species compared to those whose shells are comprised only of aragonite (Gardner *et al.*, 2018). Regardless of the mechanisms involved, our data would support the hypothesis that marine calcifiers differ in their sensitivity to OA, with larval stages being more sensitive than later-life history stages. Considering model projections that predict occasional $\Omega_{\text{arg}} \sim 1$ and seasonal $\Omega_{\text{arg}} < 1$ conditions by 2030 and 2080 respectively in the region (Artoli *et al.*, 2014; Ostle *et al.*, 2016), the Stonehaven monitoring site represents a unique natural laboratory to examine the biological impact of OA on coastal systems.

Value of time-series on assessing potential impacts of OA

The need for sustained concomitant biological and chemical observations has been recognized by the marine science community as a critical point to understand the potential impact of OA on marine systems (IPCC, 2014; Ostle *et al.*, 2016; Manno *et al.*, 2017). However, long-term monitoring on OA for coastal waters and shelf seas are currently lacking (Williamson and Widdicombe, 2018), constraining our understanding of temporal-spatial responses to OA and our capability to determinate long-term trends. This study is, to our knowledge, one of the few investigations providing baseline information about the temporal variability of the potential impacts of OA on pelagic gastropods. These types of studies illustrate the value of time series to provide the evidences required to fill those knowledge gaps (e.g. to interpret data collected during sporadic cruises). They also highlight the need for long-term scale monitoring to better inform policy makers and marine industries on OA and biological impacts and to evaluate adaptation and mitigation strategies (Hurd *et al.*, 2018).

Conclusions

This study examines the shell integrity of pelagic gastropods routinely observed in one of the few sustained monitoring sites of OA and plankton in coastal waters in the NE Atlantic. This study provides, to the best of our knowledge, the first description of the variation in shell dissolution of shelled pteropods and pelagic larval stages of benthic gastropods in a biological time-series, and attempt to explore the relationships with environmental variables and carbonate chemistry. Evidence of shell dissolution were observed in all analysed taxa despite the seawater being supersaturated with respect to aragonite, supporting previous studies indicating that dissolution may appear under higher saturation values than previously assumed. Temporal variation in shell condition matched carbonate chemistry patterns, particularly seasonal and sporadic decreases in aragonite saturation levels. Our data also support the hypothesis that marine calcifier response to OA may differ even across closely related taxonomic groups. The pronounced sensitivity of early life stages suggests that larvae may be more sensitive indicators of OA effects than adult stages. The temporal variability of shell dissolution and carbonate chemistry illustrates the complexity of the analysis of the potential impacts of OA in coastal systems. Longer time series of data will be required to elucidate these relationships further.

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