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Project: Can pelagic gastropods be used to assess the impacts of ocean acidification?

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Note
The dissemination of the results described in the present report includes the production of peer-review manuscripts. Please, may the reader be aware of this when disseminating this report.

Introduction

Levels of atmospheric carbon dioxide are increasing at unprecedented rates due to anthropogenic activities. More than a third of this carbon dioxide is taken up by the ocean lowering the pH in seawater (Ocean Acidification – OA). This process and the associated alteration to seawater carbonate chemistry are likely to have a significant impact on plankton calcifiers, which build their shells from chemical forms of calcium carbonate (calcite and/or aragonite), as shell development becomes more energetically costly (Gazeau et al., 2013). Initially most planktonic studies investigating the impacts of OA focused on coccolithophores with mixed results (Meyer & Riebesell, 2015), while other calcareous groups (such as marine shelled molluscs) have received less attention (Doney et al., 2009). Marine shelled molluscs play an important role in the marine environment e.g. providing habitat structure for benthic organisms and food sources for other organisms (Gazeau et al., 2013). This group is currently receiving considerably more attention from the international scientific community with an increasing number of reports and papers focusing on the potential impact of OA on pelagic gastropods (Gazeau et al., 2013).

Pelagic gastropods are shelled molluscs (e.g. "Green ormer", *Haliotis tuberculata*). This group includes the pelagic larvae of otherwise benthic gastropod species (e.g. "violet snail", *Janthina janthina*) as well as holoplanktonic pteropods (such as "sea butterflies") whose entire life cycle is planktonic. Pelagic gastropods are widely distributed in the world's oceans. They represent an important component of pelagic food webs linking phytoplankton and large pelagic predators such as zooplankton, fishes, cephalopods, and birds (Lalli and Gilmer 1989; Suárez-Morales et al. 2009). Pelagic gastropods also play an important role in biogeochemical cycles of the ocean. Their shells, comprised of aragonite, sometimes calcite and in certain taxa layers of both calcite and aragonite (Addadi et al., 2006), act as a vehicle of rapid transport of carbon to the ocean interior (Francois et al., 2002).

Available literature on benthic gastropods suggest that the most sensitive life-history stage to OA seems to be the pelagic larvae, with a large majority of studies on this critical stage of development reporting negative effects (e.g. Gazeau et al., 2013; Parker et al., 2013). Pteropods form their shells from aragonite. This shell mineralogy makes this group particularly susceptible to changes caused by OA since aragonite is ~50% more soluble in water than calcite (Fabry et al., 2008), and thus is more sensitive to dissolution under acidified conditions (Comeau et al., 2010; Maas et al., 2012). Because of this heightened sensitivity, pteropods may have potential to act as an indicator of OA. The genus *Limacina* is already used as an indicator of OA in the marine ecosystem along the eastern Pacific coast (Bednaršek et al., 2014), however no work has been performed in UK waters and little is known about the diversity of this group in this region. The response of pelagic gastropods to OA may be taxon-specific (Gazeau et al., 2013; Bednaršek et al., 2016) and thus more information is required before the use of this group as a potential indicator of OA in the marine environment can be considered.

Marine Scotland Science (MSS) operated coastal ecosystem monitoring site at Stonehaven (56º 57.8′ N, 02 º 06.2′ W) is one of the few time series in the North East Atlantic where pelagic gastropods are collected on a weekly basis. Between 2009 and 2013 (Walsham et al., 2015), water samples have been analysed weekly for carbonate chemistry parameters "Total Alkalinity" (TA) and "Dissolved Inorganic Carbon" (DIC). Shell dissolution and diversity in pelagic gastropods has yet to be examined in detail at the Stonehaven monitoring site.

The aim of this project is to investigate if shell dissolution can be seen in pelagic gastropods shells from the Stonehaven monitoring site and if this is related to the carbonate chemistry in the water column. This will be achieved by:

(1) Scanning Electron Microscopy (SEM) of pelagic gastropod species collected at Stonehaven to identify shell dissolution on shells.

(2) Assessing the elemental composition of shells from different gastropod species using X-ray microanalysis.

(3) Identification of the pelagic gastropod larvae and pteropods present at the Stonehaven monitoring site, using the SEM results and molecular analysis.

(4) Investigation if temporal shell dissolution or elemental composition patterns are associated with carbonate chemistry and/or environmental parameters.

(5) Evaluation of the potential use of pelagic gastropods shell dissolution in the investigation of the impacts of OA on the plankton community in the Northern North Sea.

Material and methods

Monitoring site

The Stonehaven monitoring site is located 5 km offshore from the village of Stonehaven in the North East of Scotland (Figure 1) and is 50 m deep. It is part of the MSS coastal ecosystem monitoring programme. Temperature, salinity, nutrients, phytoplankton and zooplankton abundance has been performed weekly (weather permitting) since monitoring began in 1997. Data from the Stonehaven monitoring site is supplied to fulfil the monitoring requirements of the Water Framework Directive and Marine Strategy Framework Directives.

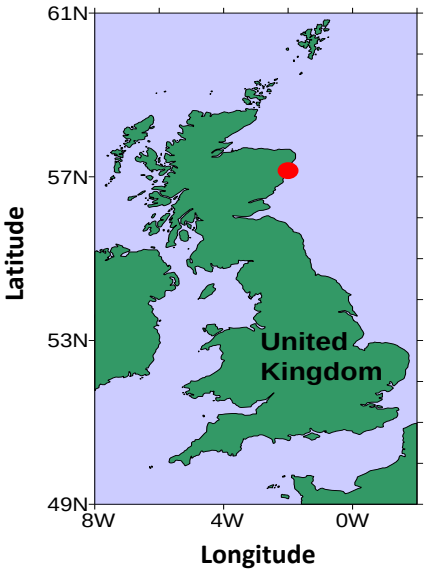

Figure 1. Location of Stonehaven monitoring site.

Water samples for salinity and nutrients were obtained at 1 m and 45 m depth using Niskin sampling bottles. The latter were fitted with digital thermometers to record temperature at each sampling depth. Salinity samples were analysed using a Guildline Portasal Salinometer Model 8410A, previously standardised using IAPSO standard seawater. Inorganic nutrient concentration was determined by segmented flow analysis using a Bran-Luebbe QuAAtro autoanalyser. Water samples for chlorophyll "a" (Chl a) were obtained using a 10 m Lund tube, providing and integrated sample of the water column. 1 L of seawater was filtered through GF/F glass fibre filters and store frozen at -80 ºC until analysis. Chl a concentration was determined by fluorometry after extracting the pigments in buffered acetone for 24 h. (Bresnan et al., 2015). Zooplankton samples were collected by vertical hauls from 45 m to surface with a Bongo net (68 μm mesh, 0.40 m diameter) and preserved in ethanol until analysis under the SEM. A full description of the methodology used at the Stonehaven monitoring site is detailed in Bresnan et al., 2015.

Additional water samples were collected weekly between 2009 and 2013 for the determination of the carbonate chemistry parameters; TA and DIC. Samples for TA and DIC were analysed on a Marianda Vindta 3C based on the procedures of Dickson (2007). The pH and the calcite and aragonite saturation constants were derived using CO_2SYS (version 2.1).

SEM analysis

Pelagic gastropod specimens were isolated from archived zooplankton samples collected monthly between 2011-2013 at Stonehaven and subsequently examined using a Zeiss EVO MA10 SEM at the Institute of Medical Science at the University of Aberdeen. The method described by Bednaršek et al. (2012) to obtain clean pteropod shell surfaces was tested. This method revealed a number of issues relating to the chemicals used. After consultation with the author an amended version of the protocol was tested with satisfactory results (this method is supplied in the appendix section). Prior to SEM analysis, shells were carefully mounted on aluminium SEM stubs using fine brushes and gold coated under vacuum conditions.

Typically, the pteropod genus *Limacina* has a shell microstructure consisting of thick underlying cross-lamellar layers and an upper thin prismatic layer that is covered with an organic layer (periostracum) (Bednaršek et al., 2012). In this study we assumed a similar structure for gastropod larvae shells although further research is ongoing to know if other structures are present in our samples.

SEM images from each specimen were used to identify shells and determine if mechanical or dissolution damage was present. If any dissolution was present, specimens with non-mechanical shell damage were categorized into three levels of

dissolution based on the degree of shell damage using criteria defined in Bednaršek et al. (2012) (Figure 2): Type I, partial dissolution of the prismatic layer; Type II, partial disappearance of the prismatic layer and exposure of the crossed-lamellar layer; Type III, signs of dissolution of the crossed-lamellar layer. The last two types indicate severe damage on shell integrity (Bednaršek et al., 2012).

A semi-automated software (Bednaršek et al., 2012) was tested as an assistance tool for the categorization of shell dissolution. First trials of the software highlighted several issues related to errors in the computation code (developed in Matlab). After consultation with the author, the image analysis was carried out manually using the categorization criteria defined above (Bednaršek et al., 2012).

Additional X-ray microanalysis were performed with an Oxford INCA microanalysis detector to look at elemental composition of shells and to assess potential changes in the elemental composition associated with dissolution of the integrity of those shells (Figure 3).

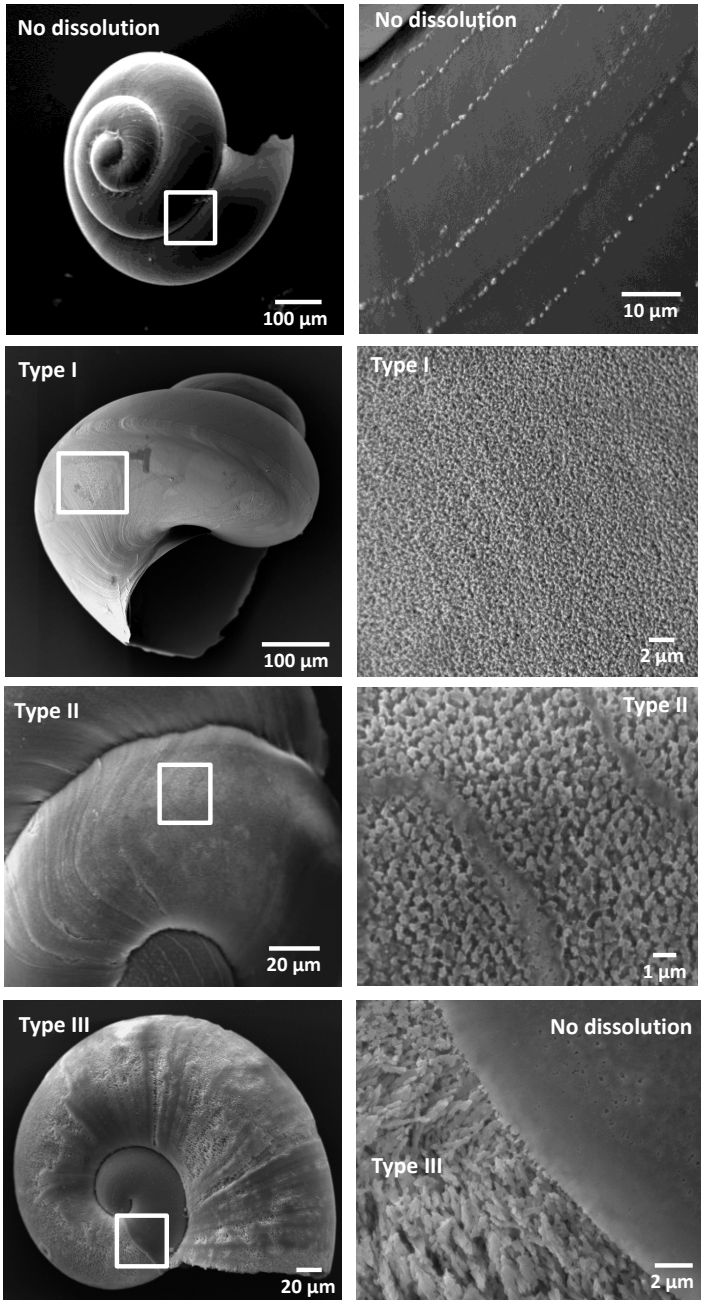

Figure 2. SEM pictures showing shell dissolution categories according to Bednaršek et al. (2012).

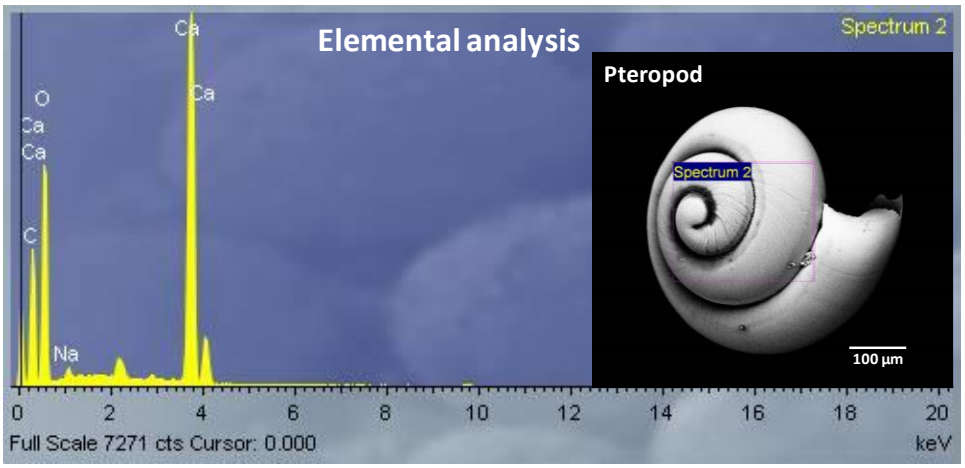

Figure 3. X-ray micro analysis of pteropod shell under SEM showing its elemental composition (i.e. carbon, oxygen, sodium and calcium).

Molecular analysis

Existing methodologies (COI mtDNA) for the molecular analysis of pelagic gastropod larvae and pteropods were tested (Burridge et al., 2015). This work was funded by the Scottish Government schedule of service ST03p. The extraction of total DNA from isolated pelagic gastropod larvae from 95% ethanol preserved samples was performed using the DNeasy Blood and Tissue kit (Qiagen). Amplification of a 660 bp fragment of the COI gene was carried out using the primers LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') from Folmer et al. (1994). A polymerase chain reaction (PCR) of COI gene, using a total volume of 25 µl was optimized using the protocols described in Jennings et al. (2010) and Burridge et al. (2015). PCR products were purified using illustra ExoProStar and sent for sequencing to the DNA Sequencing & Services, College of Life Sciences, University of Dundee, Dundee, Scotland. The genus-level identification of sequences was confirmed by BLAST searching GenBank (Altschul et al., 1997). Early results show the suitability of the procedure carried out for total DNA extraction, selection and amplification of primers, and identification of sequences to investigate pelagic gastropod identity. This work will be finished by MSS during 2016.

Statistical analyses

Incidence of shell dissolution was compared with carbonate chemistry and environmental data from the same period. For each month, mean values of temperature, salinity, Chl a, pH, calcite and aragonite saturation coefficient were calculated. Pearson correlations were performed to test for similarities between the different variables. A Principal Component Analysis was performed to research the relationship among carbon chemistry-environmental parameters and shell

dissolution. The software package Statistica 7.1 (Statsoft, Inc. 1984-2005) was used for the statistical analyses.

The project objectives and early outcomes haven been already presented at a number of national and international forums, including the "Response of pteropods to ocean acidification and climate change" Workshop (Cambridge, June 2015), ICES Annual Science Conference (Copenhagen, September 2015), the Marine Alliance for Science and Technology in Scotland (MASTs) Annual Science Meeting (Glasgow, September 2015) and the ICES/PICES "6th Zooplankton Production Symposium" (Bergen, May 2016). Dissemination of the results will also include the production of at least one manuscript for peer review during 2016. These abstracts are included in an appendix.

Results

Environmental variables and zooplankton

Seasonal variability of environmental parameters at Stonehaven is shown in Figure 4. Temperature followed a typical seasonal cycle with increasing values from late spring to late summer/early autumn and colder waters in winter. Salinity showed a higher interannual variability, with an increase observed during September – October 2012 suggesting an influence from offshore waters (Figure 4A) (Walsham et al., 2015). Chlorophyll concentration was low from late autumn to late spring/early summer months when it increased. pH values were higher between August-December 2011 and decreased during the rest of the study period, except in April 2012 and May 2013 (Figure 4B) when they peaked coinciding with the beginning of the phytoplankton bloom. Calcite and aragonite saturation levels ranged from 2.48-3.52 and from 1.56-2.25 respectively (Figure 4C), indicating that seawater at Stonehaven was oversaturated respect to both chemical carbonate forms during the study period.

Zooplankton data from the Stonehaven monitoring site since 1999 showed that pelagic gastropod larvae peaked in abundance during April and September while pteropods had a peak abundance in September (Figure 5). Pelagic gastropod larvae were more abundant than pteropods at this site.

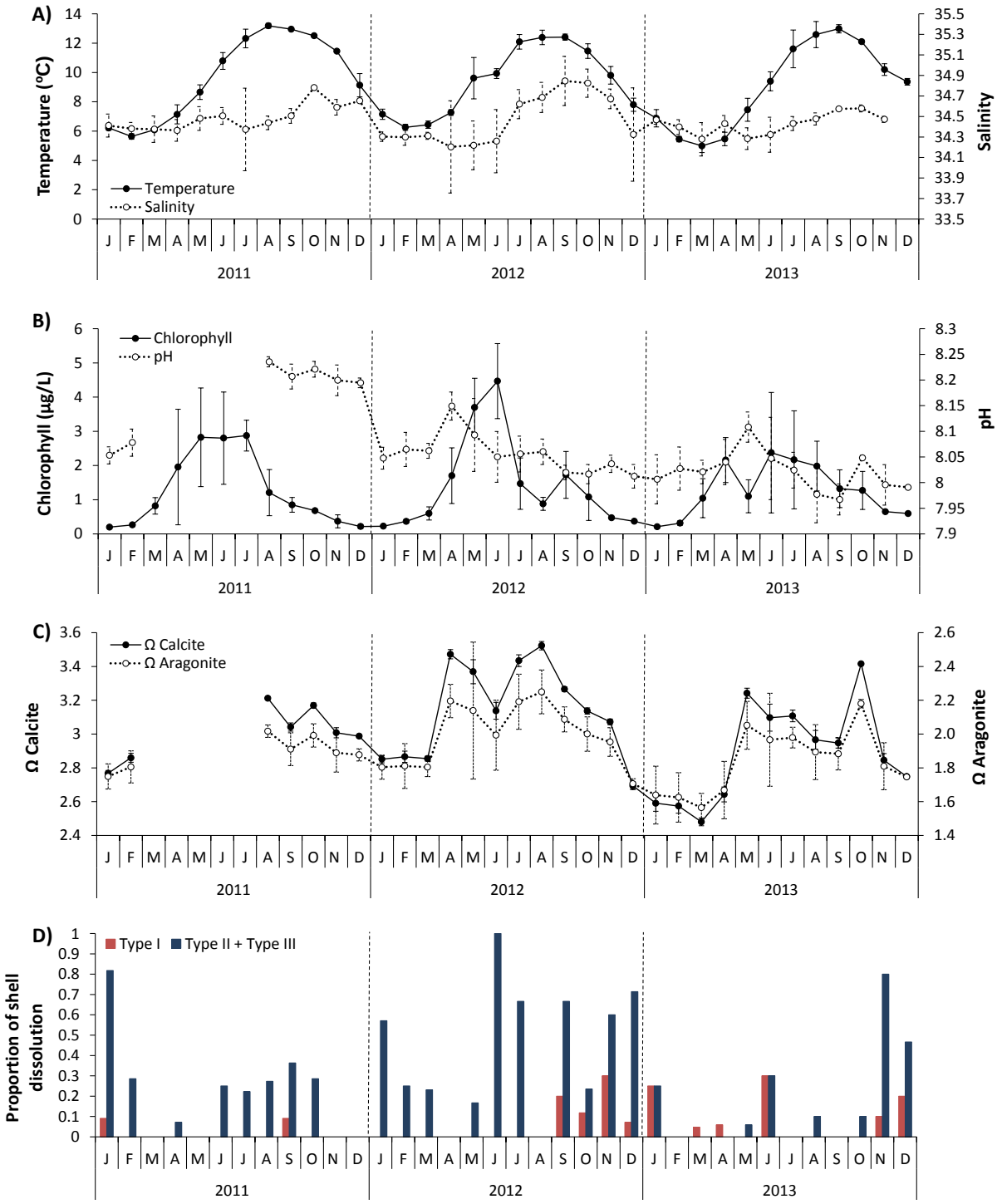

Figure 4. Seasonal variability of (A) temperature, salinity, (B) Chlorophyll, pH, (C) Ω calcite, Ω aragonite and (D) proportion of pelagic gastropod shell dissolution at the Stonehaven monitoring site during 2011-2013. Type II and III are plotted together since both indicate severe damage on shell integrity.

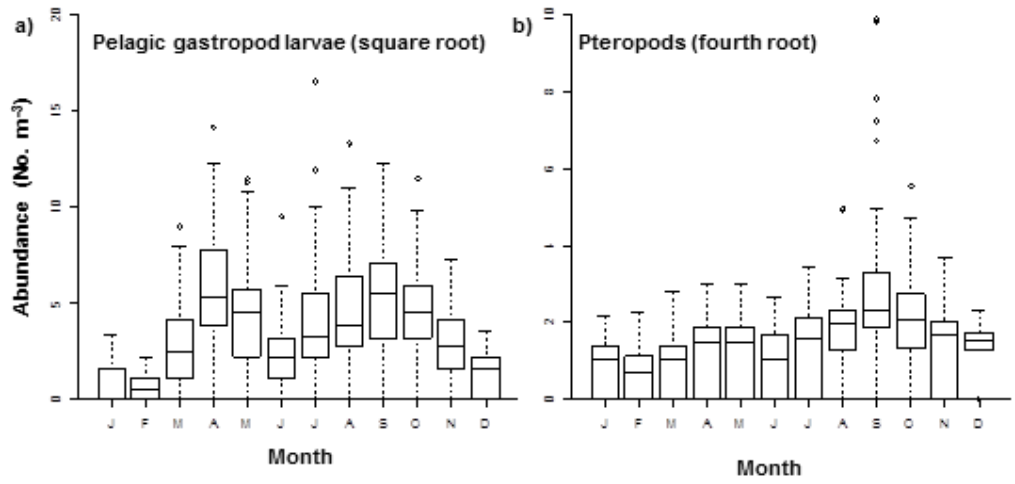

Figure 5. Monthly average of gastropod larvae (a) and pteropods (b) from Stonehaven from 1999 to 2013.

Evidence of shell dissolution

Seventy three individuals (~16%) of the 450 isolated specimens showed mechanical damage on shells and were not considered further for SEM analysis. SEM data showed evidence of shell dissolution in 125 of the 377 shells (29 pteropods and 348 gastropods) examined (approx. 1/3 of all studied); 22 Type I, 52 Type II and 51 Type III. The analysis of shell dissolution during the study period showed a high variability between the different years (Figure 4D). A seasonal pattern was less clear, with a greater proportion of damaged shells observed during the winters in 2012 and 2013 while evidence of shell dissolution was also observed during summer in 2011 and 2012 (Figure 4D). The proportion of damaged shells was significantly higher in pelagic gastropod larvae (121 shells) than in pteropods (Figure 6), which only showed evidence of shell dissolution in 4 specimens collected in August 2011, November 2012 and December 2013 (Figure 6). The comparison of temporal dissolution with environmental data (temperature, salinity and chlorophyll) and carbon chemistry collected at Stonehaven from the same period did not reveal any particular association with environmental parameters (Figure 4). Evidence of shell dissolution was observed under colder and fresher waters during the winter in 2012-2013 as well as under high temperature and salinity in summer 2011-2012. Shell dissolution also did not follow the chlorophyll seasonal pattern. While the proportion of shell dissolution was higher in the winter of 2012 coinciding with a decrease of pH and calcite-aragonite saturation values this was also observed in June 2013 when both parameters peaked. Statistical tests did not show significant relationship among evidence of shell dissolution and environmental parameters.

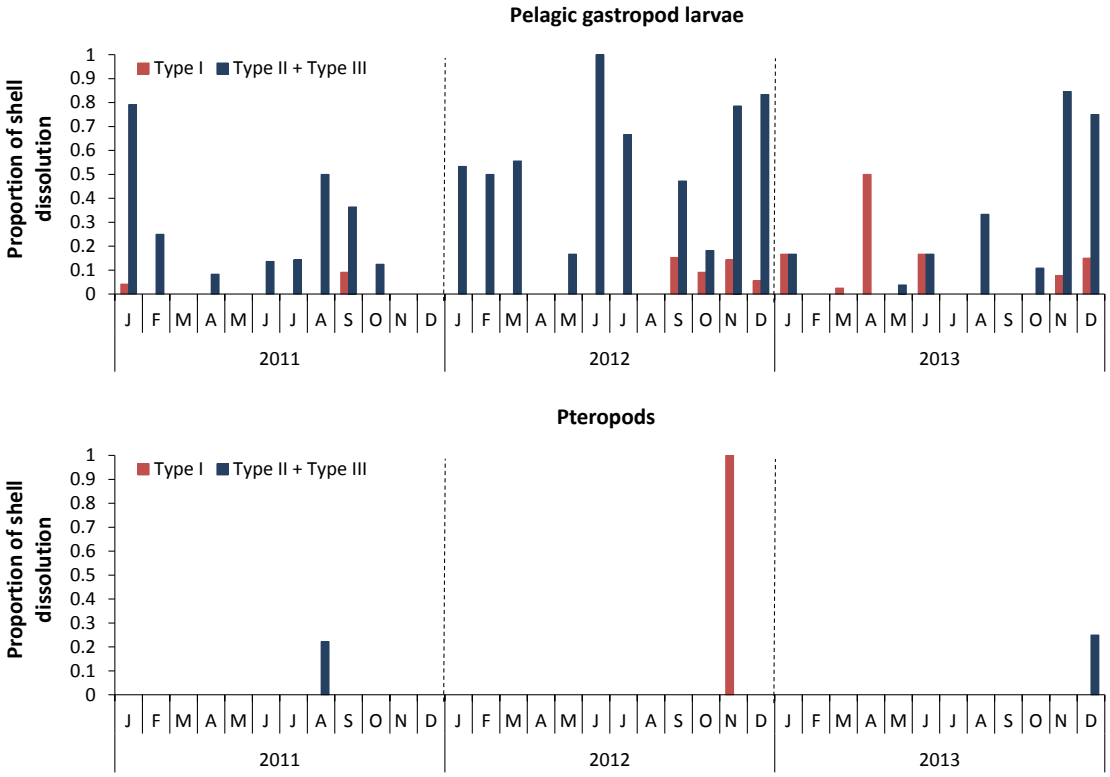

Figure 6. Seasonal variability of proportion of shell dissolution at the Stonehaven monitoring site during 2011-2013. Top: Pelagic gastropod larvae; bottom: pteropods. Type II and III are plotted together since both indicate severe damage on shell integrity.

Elemental analysis

Concomitant X-ray microanalysis of the specimens (Table 1) revealed oxygen, calcium and carbon as the main components of the shells, followed by other elements such as copper, sodium, chlorine, potassium aluminium, silica, magnesium, and sulphur. Some other elements like iron and titanium were also present in some shells but in minor percentages. The comparison of damaged and non-damaged areas of the shells did not show significant differences in the percentage of the main components (carbon, oxygen and calcium) (Figure 7).

Element	Weight (%)
Oxygen	55.90
Calcium	41.82
Carbon	14.90
Copper	5.41
Sodium	0.80
Chlorine	0.29
Potassium	0.11
Aluminium	0.06
Silica	0.06
Magnesium	0.04
Sulphur	0.02
Iron	0.01
Titanium	-

Table 1. Elemental composition of shells obtained from X-ray analysis.

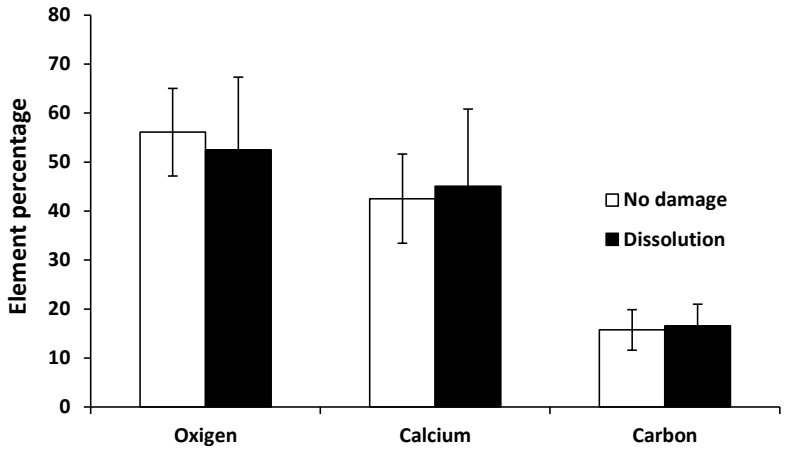

Figure 7. Average percentage of oxygen, calcium and carbon on both undamaged and shells with evidence of dissolution.

Identification of pelagic gastropod larvae and pteropods

SEM images revealed specimens with different shape, size and shell ornamentation (Figure 8), suggesting the presence of two species of pteropods (*Limacina helicina* and *Limacina retroversa*) and several species of pelagic gastropods (possibly *Alvania* ***spp.***, *Littorina* spp., *Rissoa spp.*, *Aporrhais* spp., *Nassarius* spp., among others) although taxonomic literature and expertise on this group is very scare. Molecular analyses confirmed the identity of the pteropods obtained from SEM examination. This represents the first identification of the polar *L. helicina* in Scottish waters. Molecular data also indicated the presence of the gastropod genus *Pusillina*.

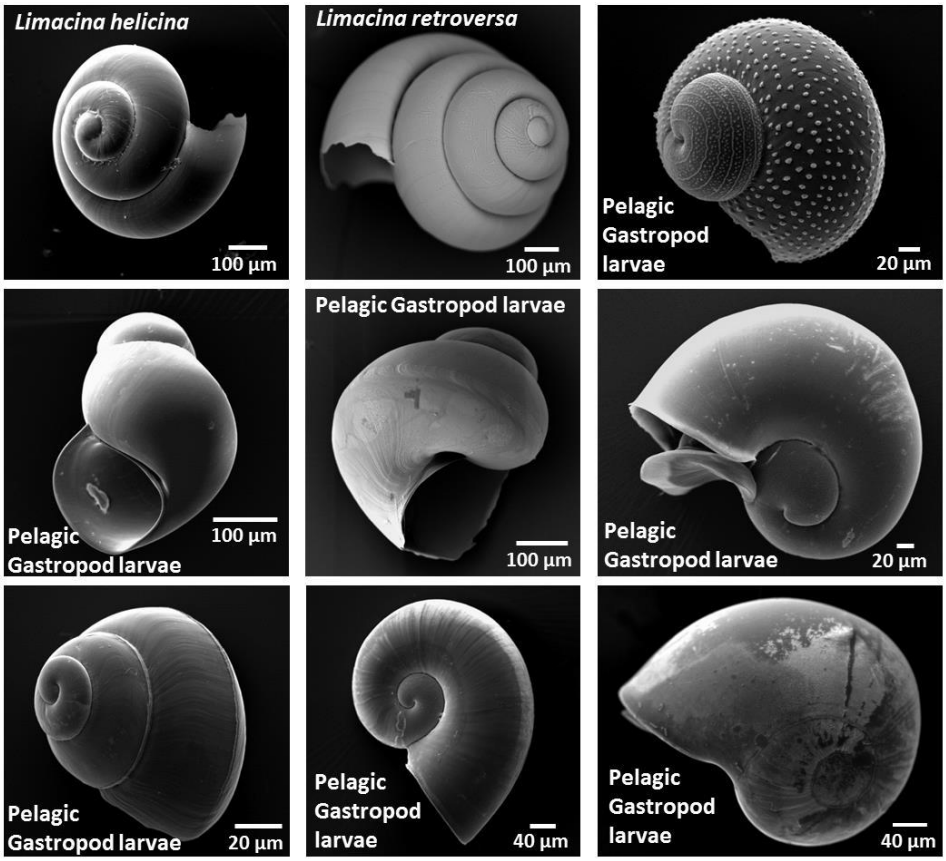

Figure 8. SEM pictures of pelagic gastropod larvae and pteropods found at the Stonehaven monitoring site.

Discussion

SEM analysis was used to examine the shells of pelagic gastropods at the Stonehaven monitoring site. This analysis, covering 3-years samples corresponding to the period 2011-2013. Despite both seawater aragonite and calcite saturation were not a limiting factor for calcification shell dissolution was observed with almost one third of shells examined presenting evidence of dissolution. A higher incidence of dissolution was observed in gastropod shells than in pteropods (34.5% and 13.8% of specimens respectively) which suggest pelagic gastropod larvae may be more sensitive indicators.

The comparison of shell integrity with environmental data (including temperature, salinity and chlorophyll) and carbonate chemistry collected at Stonehaven from the same period suggest a lack of relationship between environmental conditions on shell dissolution between 2011-2013. This time period is very short and a longer time series of data may be required to elucidate this relationship further. It is also not clear what time span the shell dissolution occurred over. If this took place over a period of weeks or months, the relationship with environmental variables would be difficult to tease out. Similar situations have been described in Australian tropical

waters (Roger et al., 2012) and the California Current System (Bednaršek & Ohman, 2015). These studies highlight the importance of environmental conditions experienced by the shells prior to sampling (e.g. exposure to a lower saturation state during an earlier growth stage) since dissolution could come from previous months or areas. The effects of OA on calcifying zooplankton in the published literature (e.g. Gazeau et al., 2013; Bednaršek et al., 2016) are based on continuous exposure to those conditions while the impact of natural seasonal/diurnal variability has yet to be investigated. Different taxa may not be equally sensitive to ocean acidification and responses to OA can differ by life history stage yet the taxonomy, particularly of pelagic gastropod larvae, is not well described. SEM data were used to investigate the identity of the pelagic gastropod larvae and pteropods present at Stonehaven, suggesting the presence of several species. Molecular analyses (COI mtDNA; Burridge et al., 2015) confirmed the identity of the pteropods *L. helicina* and *L. retroversa*. This is the first time that the presence of both species has been confirmed in Scottish waters. This is interesting since *Limacina helicina* is the dominant pteropod in polar waters (temperature of -1.6 to 4 ºC; maximum 7 ºC) whereas *Limacina retroversa* is present in high abundance in the sub polar areas (at water temperatures of 2–19 ºC; optimum 2–7 ºC) (Bé & Gilmer 1977; van der Spoel & Heyman 1983). SEM results suggest the presence of several species of pelagic gastropods at the Stonehaven monitoring site however lack of taxonomic literature and expertise means identification is still underway. This study flags an important 'knowledge gap' as the calcite and aragonite content of pelagic gastropod larvae and pteropod shells may vary with species an accurate assessment of the diversity of gastropod larvae is key to understand the impacts of OA.

Concomitant X-ray microanalysis of the specimens has provided the first information about the elemental composition of shells from different gastropod species at Stonehaven. It has also allowed any potential changes in the elemental composition associated with dissolution of the integrity of those shells to be assessed. Our analysis indicates no changes on elemental composition associated with shell dissolution in pelagic gastropods in the region, in contrast with similar analyses carried out on pteropods collected in the California Current System (Bednaršek, pers. comm.).

Conclusions

The methodology to study shell dissolution in marine pelagic pteropod larvae and pteropods was optimised for use in samples from the Stonehaven monitoring site.

Evidence of shell dissolution in marine pelagic gastropod larvae and pteropods was observed in 33% of shells. This is the first record of shell dissolution in Scottish waters.

SEM revealed the pelagic gastropod larvae to be diverse but taxonomic expertise in this group is scarce and work is still underway to confirm identifications. This 'knowledge gap' may hinder future work in this area.

Molecular methods confirmed the presence of *L. retroversa* and *L. helicina* at Stonehaven and provides the first record of the polar *L. helicina* in Scottish waters.

Relationship between shell dissolution and environmental variables is complex and further work is required to establish the relationship with carbonate chemistry.

No change in elemental composition of shells with dissolution observed.

Recommendations to ICES

The lack of taxonomic information and proper identification keys to identify marine pelagic gastropod larvae using morphological criteria limits the ability to properly assess the impacts of OA on this group. A taxonomic identification leaflet of key species in the ICES area would be a valuable resource to forward this area of research.

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Appendix.

Alternative approach for shell preparation for SEM analysis.
The method described by Bednaršek et al. (2012) allows obtaining clean and intact shells surfaces suitable for SEM examination through several steps to i) remove the abiogenic crystals from shell surface, ii) dehydration of shells and iii) the removal of the organic layer (periostracum). This method has been recently amended (Bednaršek et al., 2016) by replacing the original procedure for the removal of the periostracum with and easier and more readily available chemical treatment: the use of 6% hydrogen peroxide solution followed with a 1% KOH.

The detailed cleaning process is as follows:

1. Shells are washed with 70% ethanol for 2-3 minutes.
2. Samples are subsequently cleaned with 50% ethanol for 2-3 minutes.
3. Shells are washed twice with distilled water for 5 minutes.
4. Samples are treated with 2 consecutive rinses in 6% hydrogen peroxide for 20 minutes.
5. Shells are washed twice with distilled water for 5 minutes.
6. Shells are then immersed in 1% KOH for 2 hours for removal of organic layer.
7. Shells are finally washed twice with distilled water for 5 minutes.
8. Shells are mounted on aluminium stubs using and air dried until analysis gold-coating for examination under the SEM.

Bednaršek, N., Johnson, J., Feely, R.A. 2016. Comment on Peck et al: Vulnerability of pteropod (*Limacina helicina*) to ocean acidification: shell dissolution occurs despite an intact organic layer. Deep Sea Research II 127, 53-56.

Poster presentations.

- Abstract submitted to the "Response of pteropods to ocean acidification and climate change" Workshop (Cambridge, June 2015).

Can pelagic gastropods be used to assess the impacts of ocean acidification in the North Sea?

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Ocean acidification (OA) and the associated alteration to seawater carbonate chemistry is likely to have a significant impact on plankton calcifiers. However laboratory experiments have shown mixed results and field studies are scarce.

At the Marine Scotland Science (MSS) Stonehaven ecosystem monitoring site on the east coast of Scotland (56 57.80N, 02 06.20W), temperature, salinity, nutrient concentrations and plankton abundance have been measured weekly since 1997. Since 2009, water samples have been analysed weekly for carbonate chemistry parameters Total Alkalinity (TA) and Dissolved Inorganic Carbon (DIC) and monthly samples have been examined since 2010 to assess the diversity of coccolithophores present. This MSS programme is providing baseline information about the seasonality and interannual variability of TA and DIC as well as coccolithophores in inshore waters at this site.

An investigation of other biological components potentially threatened by OA, the pelagic gastropods, will be presented. This study involves Scanning Electron Microscopy (SEM) and X-ray microanalysis of pelagic larvae of benthic gastropod species and holoplanktonic pteropods collected at Stonehaven to detect any evidence of shell dissolution and to determinate the elemental composition of shells. These results will be compared with concomitant environmental/carbonate chemistry data to evaluate if any temporal dissolution or elemental composition patterns can be associated with OA and/or seasonal or environmental parameters. We intend to evaluate if the analysis of pelagic gastropods shells can be used as a tool to assess the impacts of OA on the plankton community in the Northern North Sea.

Keywords: Ocean Acidification; Gastropods; Pteropods; North Sea; "Impact Assessment".

- Abstract submitted to the "ICES Annual Science Conference (Copenhagen, September 2015).

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Keywords: Plankton; Ocean Acidification; Gastropods; North Sea; "Impact Assessment".

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- Abstract submitted to the "Marine Alliance for Science and Technology in Scotland (MASTs) Annual Science Meeting (Glasgow, September 2015).

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- Abstract submitted to the "PICES/ICES 6th Zooplankton Production Symposium" (Bergen, May 2016).

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Can pelagic gastropods be used to assess the impacts of ocean acidification in the North Sea?

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The potential impact of ocean acidification (OA) on plankton calcifiers is a focus of interest for the marine science community. Most planktonic studies have focused on coccolithophores with mixed results, while other calcareous groups of great ecological significance have received less attention. This study will present the first investigation of the impacts of OA on pelagic gastropods at the Marine Scotland Science (MSS) monitoring site at Stonehaven (east coast of Scotland). Temperature, salinity, nutrients, phytoplankton and zooplankton have been monitored at the site weekly since 1997. Carbonate chemistry measurements total alkalinity (TA) and dissolved inorganic carbon (DIC) began in 2009. During this study the dissolution and elemental composition of archived pelagic gastropods shells (including pelagic larvae of benthic gastropod species and holoplanktonic pteropods) were examined using Scanning Electron Microscopy (SEM) and X-ray microanalysis. TA and DIC showed a seasonal pattern with considerable interannual variability and appeared to be influenced by higher salinity water entering the Scottish Costal system at the end of 2012. Calculated pH values were influenced by the phytoplankton biomass in the water. Incidence of gastropod shell dissolution was scored and the relationship with OA and other environmental parameters investigated. The SEM images and concomitant molecular analysis will provide the first detailed assessment of the diversity of the pelagic gastropod community at the Stonehaven monitoring site. We intend to evaluate if the analysis of pelagic gastropods shells can be used as a tool to assess the impacts of OA on the plankton community in the Northern North Sea.

Keywords: Plankton; Ocean Acidification; Gastropods; North Sea; "Impact Assessment".
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