ICES Journal of Marine Science

ICES Journal of Marine Science (2022), 79(3), 963–973. https://doi.org/10.1093/icesjms/fsab263

Original Article

Investigations into the relationship between domoic acid and copepods in Scottish waters

Kathryn B. Cook [1,2,*], Jean-Pierre Lacaze[2], Margarita Machairopoulou[2], and Eileen Bresnan[2]

[1]National Oceanography Centre, European Way, Southampton SO14 3ZH, UK
[2]Marine Scotland Science, Marine Laboratory, 375 Victoria Road, Aberdeen AB11 9DB, UK

*Corresponding author: tel: +44 (0)23 8059 8487; e-mail: Kathryn.Cook@noc.ac.uk.

Cook, K. B., Lacaze, J.P., Machairopoulou, M., and Bresnan, E. Investigations into the relationship between domoic acid and copepods in Scottish waters. – ICES Journal of Marine Science, 79: 963–973.

Received 14 June 2021; revised 6 December 2021; accepted 8 December 2021; advance access publication 20 January 2022.

This study investigated impacts of the algal toxin domoic acid (DA) on copepods in Scottish waters. Inspection of seasonal patterns revealed that several common copepods (*Acartia* spp. Dana, 1846, *Calanus* spp. Leach, 1816, *Centropages* spp. Krøyer, 1849, *Pseudocalanus* spp. Boeck, 1872, and *Temora longicornis* (Müller O.F., 1785)) regularly coexist with potentially toxic species from the diatom genus *Pseudo-nitzschia* H. Peragallo in H. Peragallo and M Peragallo, 1900. A short field study investigating the DA content of *Calanus* spp. at the Scottish Coastal Observatory site at Stonehaven recorded DA during every sampling event. The highest DA levels were associated with a July bloom (~135000 cells L^{-1}) of *Pseudo-nitzschia* cf. *plurisecta* Orive & Pérez-Aicua 2013. Several studies have previously investigated effects of ingested DA on copepods but information on effects of dissolved DA is lacking, therefore, simple exposure experiments were carried out to measure mortality of copepod species at ecologically relevant concentrations of dissolved DA. The highest concentrations tested ($\geq$ 50 ng DA mL^{-1}) decreased survival in *Temora longicornis* only; survival of other copepod species was unaffected. However, *T. longicornis* feeding on non-toxic algae in the presence of dissolved DA did not accumulate DA in their tissue. This study provides evidence of the potential for *Calanus* spp. to act as vectors for DA to higher trophic levels in Scottish waters.

Keywords: *Calanus*, copepods, domoic acid, Loch Ewe, *Pseudo-nitzschia*, Stonehaven.

Introduction

The diatom genus *Pseudo-nitzschia*, responsible for the production of domoic acid (DA), the toxin associated with Amnesic Shellfish Poisoning (ASP), has been associated with closures of shellfish harvesting areas throughout the Northeast Atlantic (Bresnan *et al.*, 2021; Karlson *et al.*, 2021). In Scottish waters, DA levels in the gonads of *Pecten maximus* above EU regulatory thresholds were responsible for wide scale closures of the Scottish Scallop fishing industry in the late 1990s (Gallacher *et al.*, 2001). In 2005, the EU shellfish hygiene directive (now EU 2019/627) was amended to facilitate shucking and end product testing of *P. maximus*, allowing the sale of adductor muscle if DA levels were less than 4 mg kg^{-1} (Bresnan *et al.*, 2017). *Pseudo-nitzschia* cells are a common component of the phytoplankton community in Scottish waters (Fehling *et al.*, 2006; Bresnan *et al.*, 2015) however their impact on their copepod grazers in this region has yet to be investigated.

Copepods form an important energy link between phytoplankton and fish stocks in marine ecosystems (Runge, 1988; Mann, 1993). As potential grazers of *Pseudo-nitzschia*, copepods can accumulate DA which can be transferred to higher trophic levels such as fish, marine mammals, and seabirds (see review by Turner, 2014). Records of impacts of DA on higher trophic levels in the UK have been reported suggesting they are being passed up the food chain. Studies have revealed the presence of DA, as well as saxitoxin (responsible for Paralytic Shellfish Poisoning) in the faeces, urine and amniotic fluid of seals (*Phoca phoca*) in Scottish waters (Hall and Frame, 2010). Evidence that DA is being passed up the food chain through consumption of contaminated fish (Jensen *et al.*, 2015) has been supported by a recent study showing that a wide range of fish

species around the Scottish coast can act as vectors for DA to top marine predators (Kershaw *et al.*, 2021).

Recent advances in metabolic profiling have led to the discovery of a new class of taurine containing polar lipids produced by copepods called copepodamides (Selander *et al.*, 2015) which have been shown to increase DA production in the diatom *Pseudo-nitzschia seriata* (Cleve) H. Peragallo, 1899. Copepodamides have subsequently been found to occur naturally in the ocean at levels observed to induce DA production in laboratory experiments (Selander *et al.*, 2019), suggesting that this interaction between DA and copepods also occurs in the field.

Pseudo-nitzschia can potentially transfer DA to copepods by one of two ways: release of dissolved toxins into the surrounding seawater (either actively or passively), or by ingestion during grazing. Most of the literature assessing the effects of toxins on copepods use live cultured phytoplankton cells in feeding experiments and, therefore, assess the effects of toxins metabolized internally. Laboratory studies investigating effects of ingested DA have found little effect on copepod grazing or reproduction (reviewed by Turner, 2014).

Field studies from the Northwest Atlantic and Pacific have recorded dissolved DA concentrations of between 3.3 and 136 ng DA mL^{-1} (see reviews by Lelong *et al.*, 2012; Bates *et al.*, 2018), and have found that dissolved DA can reduce grazing in *Euphausia pacifica* and increase mortality in the copepods *Tigriopus californicus* (Shaw *et al.*, 1997), *Temora longicornis* and *Calanus glacialis* (Windust, 1997). To date little work has been done to determine if dissolved DA can impose sub-lethal or lethal effects on copepods in Scottish waters. Reduced copepod population fitness and productivity, caused by algal toxins such as DA or other stressors e.g. climate change, can have multiple impacts on higher levels of the ecosystem e.g. result in decreased transfer of energy to higher trophic levels (Smith, 2018). Broad scale changes in the copepod community in the Northwest European Shelf have already been recorded (Bedford *et al.*, 2020) with a dramatic decline in the copepod community reported from a Scottish sea loch (Wells *et al.*, in press). Thus, a better understanding of the effect of environmental stressors on copepods is important in order to better predict the potential consequences of environmental changes.

To examine the influence of toxin producing *Pseudo-nitzschia* spp. on the role of marine copepods as a vector for algal toxins, data from the Scottish Coastal Observatory (SCObs) time series at Stonehaven and Loch Ewe were reviewed. The seasonality of key copepod genera at both sites were examined in relation to the presence of *Pseudo-nitzschia* species. To investigate if copepods can act as vectors for DA to higher marine trophic levels in Scottish waters, individual *Calanus* spp. were collected from the Stonehaven monitoring site and analysed for the presence of DA. Several studies have previously investigated the effect of ingested DA on copepods but information on the effect of dissolved DA is lacking. To investigate the role of dissolved algal toxins on copepods, simple exposure experiments were performed to investigate the impact of dissolved DA at potentially ecologically relevant concentrations in Scottish waters on the activity and mortality of different copepod genera.

Material and methods

Sampling site

Data from two SCObs monitoring sites contributed to this study (Figure 1). Stonehaven, the east coast site in the north western North Sea, is approximately 5 km offshore

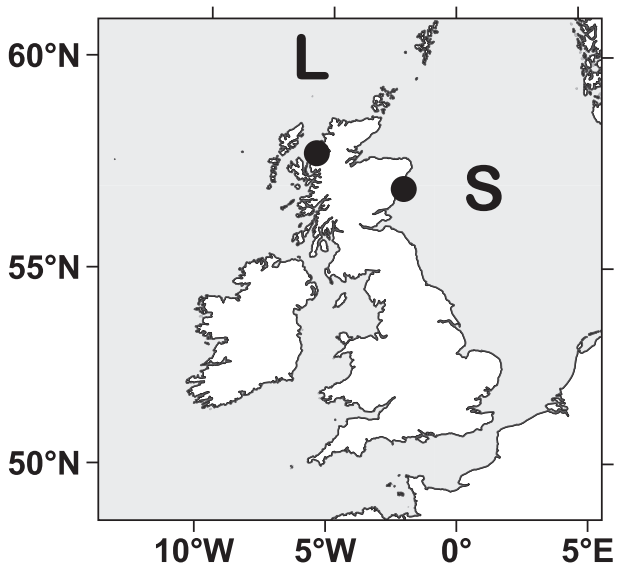

Figure 1. Marine Scotland Science coastal ecosystem monitoring sites at Stonehaven (S) and Loch Ewe (L).

(56°57.8′N 02°06.2′W) with a water depth of 50 m. Loch Ewe on the west coast of Scotland is approximately 0.5 km offshore at the mouth of a sea loch (57°50.99′N 05°38.97′W) with a water depth of 40 m. Sampling has been performed on a weekly basis (weather permitting) since monitoring began in 1997 at Stonehaven and 2002 at Loch Ewe (April 2002 for zooplankton). A description of the physics, chemistry, and plankton community at the Stonehaven and Loch Ewe sites can be found in Bresnan *et al.*, (2016).

Phytoplankton collection and analysis

Light microscopy analysis

Samples were collected with a 10 m Lund tube and a 1 L subsample was preserved immediately in 0.5% Lugol's iodine (Throndsen, 1978). On return to the laboratory, a 50 mL aliquot was settled for 48 h. *Pseudo-nitzschia* spp. cells were counted at a magnification of x200 using a Zeiss Axiovert 200 microscope using a modified Utermöhl technique (Utermöhl, 1958). *Pseudo-nitzschia* cells were separated into "*delicatissima* type" cells (diameter < 3 μm) and "*seriata* type" cells (diameter > 3 μm). Needle like *Pseudo-nitzschia* cells which were 3 μm in diameter were included in the "*delicatissima*" group. Data from the start of 2002 to the end of 2017 were used to generate a seasonal cycle for the occurrence of *Pseudo-nitzschia* at the two sites. Data is available from https://data.marine.gov.scot/group/monitoring.

Transmission electron microscopy

Integrated 68 μm mesh net haul samples preserved in 4% borax buffered formaldehyde were used to identify *Pseudo-nitzschia* cells present to species level. Due to resource limitations only samples collected from Stonehaven on 9th June 2008, 22nd July 2008, and 2nd September 2008 were analysed to investigate the diversity of *Pseudo-nitzschia* species present using Transmission Electron Microscopy (TEM). A 5 mL aliquot of the concentrated net haul sample was suspended in 45 mL of distilled water in a graduated cylin-

der and inverted to ensure the contents were well mixed. The sample was left for 15 min to allow the heavy zooplankton particles to sink to the bottom of the graduated cylinder. The top 40 mL was gently siphoned off and concentrated by centrifugation at 1500 rpm for 15 min. The supernatant was discarded and the pellet was re-suspended in distilled water, centrifuged, and re-suspended three times to rinse the formaldehyde from the sample. The remaining pellet was re-suspended in 5 mL of water and cleaned using the method described in Lundholm *et al.* (2002). A drop of the cleaned sample was loaded onto a formvar covered 200 hex grid and examined using a Joel JM 1400 Plus Transmission Electron Microscope. In total, two grids were loaded for each sample. *Pseudo-nitzschia* cells were identified using morphological criteria detailed in Skov *et al.* (1999), Lundholm *et al.*, (2002, 2003, 2006), and Orive *et al.* (2013). Each cell on the grid was identified. If fewer than 15 cells were present on the grid a second grid was examined. Samples were selected for TEM which had high cell counts using light microscopy. The *Pseudo-nitzschia* cells were not enumerated but the most abundant species on the grid was noted. In most cases, one TEM grid was examined per sample.

Zooplankton collection

Samples for the identification and enumeration of zooplankton were collected by vertical 40 cm bongo net hauls, to 45 m at Stonehaven and 35 m at Loch Ewe, using a 200 μm mesh and were preserved immediately in 4% borax buffered formaldehyde. Copepods were identified using a binocular microscope. Data from the start of 2002 until the end of 2017 were used to determine the seasonality of each group and are available from https://data.marine.gov.scot/group/monitoring.

The main copepods present during the growing season (*Acartia* spp. Dana, 1846, *Calanus* spp. Leach, 1816, *Centropages* spp. Krøyer, 1849, *Pseudocalanus* spp. Boeck, 1872, and *T. longicornis* (Müller O.F., 1785)) were selected for inclusion in dissolved DA exposure experiments. Live zooplankton for toxin analysis (during the latter half of 2008) and dissolved toxin experiments (July 2009) were collected at Stonehaven by oblique 1 m ring net tows to 45 m using a 350 μm mesh and a non-filtering cod-end to prevent damage to the animals. Fresh surface seawater was also collected for use in experiments. Live samples and seawater were transported back to a constant temperature laboratory within 2 h of collection. All handling of the samples and experiments were carried out at ambient seawater temperature (approximately 13°C). Prior to setting up each dissolved toxin experiment, at least 600 individuals of the required copepod species were carefully picked out of a live zooplankton sample and fed to excess for 24 h with Phyto Feast® Live (a mix of *Tetraselmis*, *Isochrysis*, *Pavlova*, *Nannochloropsis*, *Thalassiosira*, *Amphora*, and *Synechococcus*) and Roti-Feast® (*Brachionus plicatilis* animals and eggs) produced by Reed Mariculture.

Toxin analysis of copepods

Treatment of field copepods

Copepods were analysed for the presence of DA during 2008. Due to resource limitations this analysis was confined to the latter part of the year when toxin producing species of *Pseudo-nitzschia* were likely to be present at higher abundance (Fehling *et al.*, 2006). On each sampling occasion, 300 individual *Calanus* spp. were isolated on return to the laboratory and 100 individuals were placed into 2 mL safe lock Eppendorf tubes X3. The copepod samples were stored in a freezer at −80°C prior to extraction. *Calanus* spp. were selected for DA analysis due to their importance for fish larvae in the region (Régnier *et al.*, 2017) and because of their large body size, which would ensure that sufficient biomass was collected for toxin quantification.

Toxin analysis

For toxin analysis the method of Quilliam *et al.* (1995) was followed. A single tungsten carbide bead (3 mm diameter) was added to each safe lock Eppendorf containing the *Calanus* individuals. Extracting solvent (50% methanol) was then added with a calibrated pipette following a ratio of 100 μL/100 copepods. The copepods were homogenized for 3 min at 30 Hz using a TissueLyser 2. This was followed by centrifugation at 14000 rpm for 3 min to allow the homogenates to form a pellet. The supernatants were transferred to 0.2 μm Ultrafree-MC centrifugal filters using a pipette and this was followed by centrifugation for 10 min at 10000 rpm. The filtrates were transferred to pre-insert amber vials and were analysed straight away by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify DA. A hybrid triple quadrupole linear ion trap mass spectrometer (3200 Qtrap, AB-Sciex, Macclesfield, UK) fitted with a TurboIonSpray® ion source was coupled to a 1200 series HPLC system (Agilent, Cheadle, UK) comprising a 1200 series binary pump, a 1200 series thermostated column compartment and a 1200 series auto-sampler. Elution of DA was achieved using an Hypersil BDS C8 column (50 × 2.1 mm, 3 μm—Thermo Fisher Scientific, Altrincham, UK) using an isocratic elution (12% B). Mobile phase A was 100% aqueous and mobile phase B was 95% acetonitrile, both containing 2 mM ammonium formate and 50 mM formic acid. The flow rate was set at 0.2 mL min^{-1} and the column oven set at 20°C throughout the analysis. The injection volume for both standards and sample extracts was 5 μL. The mass spectrometer was used in multiple reaction monitoring (MRM) mode and three specific transitions, one for quantitation and the other two for confirmation, were monitored for DA. The monitored transitions and optimized source parameters are detailed in Supplementary Tables S1 and S2.

Each batch of *Calanus* spp. extracts was analysed alongside seven standards evenly interspaced between the samples. The concentrations of the DA standards ranged from 0.05 to 5.0 μg mL^{-1} and were prepared by dilution of a certified reference material, which was purchased from the National Research Council (NRC), Canada with 50:50 (v/v) methanol–water. A calibration curve was produced with each batch of samples, which was checked to ensure acceptable linearity ($R^2 > 0.99$). Toxin content of the *Calanus* spp. population at each sampling date was calculated by multiplying the toxin content per individual by the abundance (no. m^{-3}) of *Calanus* spp. determined by the routine zooplankton monitoring at the SCObs Stonehaven site.

DA exposure experiment

DA for use in copepod DA exposure experiments was purchased from Sigma-Aldrich UK. Dissolved DA concentrations have not been measured at Stonehaven, therefore, the concentrations used in the exposure bioassay were chosen to represent worst case scenarios that could arise from natural *Pseudo-nitzschia* bloom conditions in the area if 100% of the DA in cells were expelled. These were derived from published values of toxin concentrations in *Pseudo-*

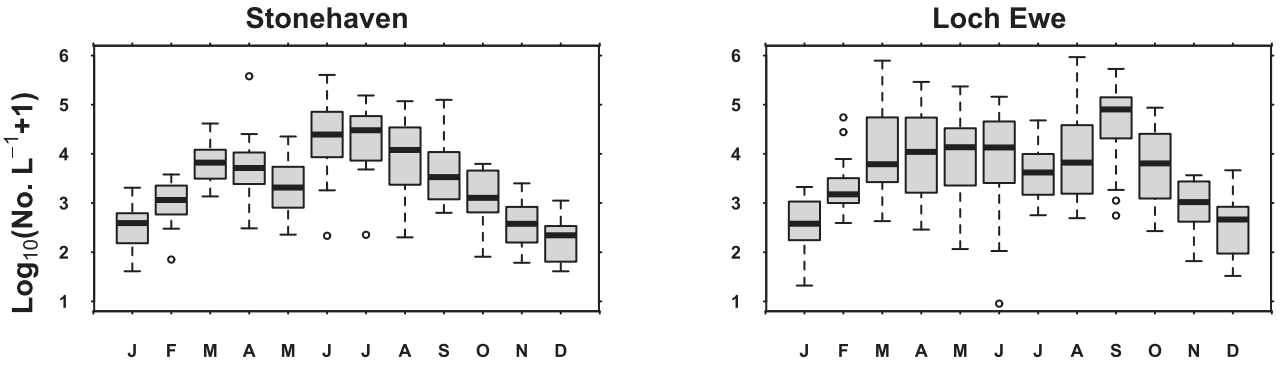

Figure 2. Box whisker plots of the monthly mean abundance of *Pseudo-nitzschia* spp. from Stonehaven and Loch Ewe 2002–2017.

nitzschia cells (Fehling *et al.*, 2004; Lundholm *et al.*, 2005; Thessen and Stoecker, 2008) and historic concentrations of *Pseudo-nitzschia* cells in the water column during the 6 years preceding the experiments. The required toxin concentrations were made up using multiple dilutions of the toxin standards with autoclaved 0.2 μm filtered seawater. A total of two different experiments were performed. In experiment 1, the DA concentrations used were 0, 0.01, 0.1, 0.5, 1.0, 2.0, 6.0, and 12 ng mL^{-1}. When results suggested some effect of DA on survival in *T. longicornis* a further exposure bioassay (experiment 2) at higher concentrations (0, 25, 50, 75, and 100 ng mL^{-1}) was performed on *T. longicornis* only in order to reach an LC_{50}.

Copepod bioassays

For each copepod species/DA concentration combination, 24 replicates of individual copepods were incubated in the dark in 3 mL of the test solution in 24-well tissue culture plates. As the copepods were not fed during the experiment, the small incubation volumes are acceptable and have been used in published copepod bioassays (e.g. Willis and Ling, 2004; Brown *et al.*, 2005; Sopanen *et al.*, 2011). The control plate was set up using autoclaved 0.2 μm filtered seawater. Each copepod was observed visually with mechanical stimulus after 1, 3, 6, 12, and 24 h to determine whether they were active (swam away from stimulus), lethargic (twitched in response to stimulus), or dead (no response to stimulus). Where an LC_{50} was not achieved after 24 h, the copepods were inspected every subsequent 24 h until 50% of the control animals were dead. Data (available in Supplementary Information) were analysed with shared frailty Cox proportional hazard models (Rondeau *et al.*, 2012) for survival data with right censoring using the survival and frailtypack libraries in the R v3.0.1 programming environment (http://www.R-project.org). The Cox proportional hazard model is a semi-parametric class of survival model that does not require a particular probability model to represent survival times. It models the effect of covariates on the hazard rate but leaves the baseline hazard unspecified and so estimates relative rather than absolute risk. Right censoring accounts for the fact that not all animals were dead at the end of the experiment, i.e. instead of knowing the exact time of death we only know death occurred at a time point after the experiment ended. Frailty modelling is an extension of survival analysis that accounts for heterogeneity and random effects. Shared frailty assumes that the random effects are common within specified groups of individuals but are random across groups. The shared frailty term was used to negate any pseudoreplication effects (Hurlbert, 1984) caused by using the wells of one culture plate as a replicate. The relationship between time to death and toxin concentration was examined for each species. The results from both *T. longicornis* experiments were analysed in one Cox proportional hazard model with experiment number added as an extra factor.

In these bioassays the copepods were starved, which could potentially reduce their filtering activity (Kiørboe *et al.*, 2018) and reduce dissolved toxin uptake. An additional grazing experiment was conducted with *T. longicornis* incubated with the non-toxic dinoflagellate *Prorocentrum micans* in medium containing DA to determine whether dissolved toxins were accumulated in the copepod body tissue during feeding. Replicate groups of three female *T. longicornis* were transferred into 75 mL bottles containing about 150 cells mL^{-1} (420 μgC L^{-1}; sufficient for maximal feeding over 24 h (Dam and Lopes, 2003)) *P. micans* and 10 ng mL^{-1} DA. Treatment bottles were incubated alongside triplicate control bottles in the dark on a rocking incubator to ensure suspension of copepods and algae. Each copepod was observed visually after 24 h to determine whether they were alive before being frozen as a single batch at −80°C for toxin analysis. The incubation water from each of the bottles was collected and preserved with acidified Lugol's iodine (1%) for enumeration of algal cells so that ingestion could be calculated using the equations of Frost (1972).

Results

Seasonality of Pseudo-nitzschia spp.

A difference in the timing and abundance of *Pseudo-nitzschia* cells between the monitoring sites on the east and west coast of Scotland was observed during 2002–2017 (Figure 2). A bimodal seasonality of *Pseudo-nitzschia* can be observed at Loch Ewe, with higher cell densities recorded at this site particularly during the spring bloom period. At Stonehaven, highest *Pseudo-nitzschia* spp. cell densities tend to be observed during the summer months. *Pseudo-nitzschia* cell counts from this monitoring dataset during the 6 years preceding the experiments, along with toxin concentrations detailed in references (see methods) were used to select the range of toxin concentrations used in the DA exposure experiment.

Seasonality of copepod genera

The copepods selected for this study (*Acartia* spp., *Calanus* spp., *Centropages* spp., *Para/Pseudocalanus* spp., and *T. longicornis*) made up 76.1% and 75.2% of the total annual copepod population at the east coast Stonehaven and west coast Loch Ewe monitoring sites,

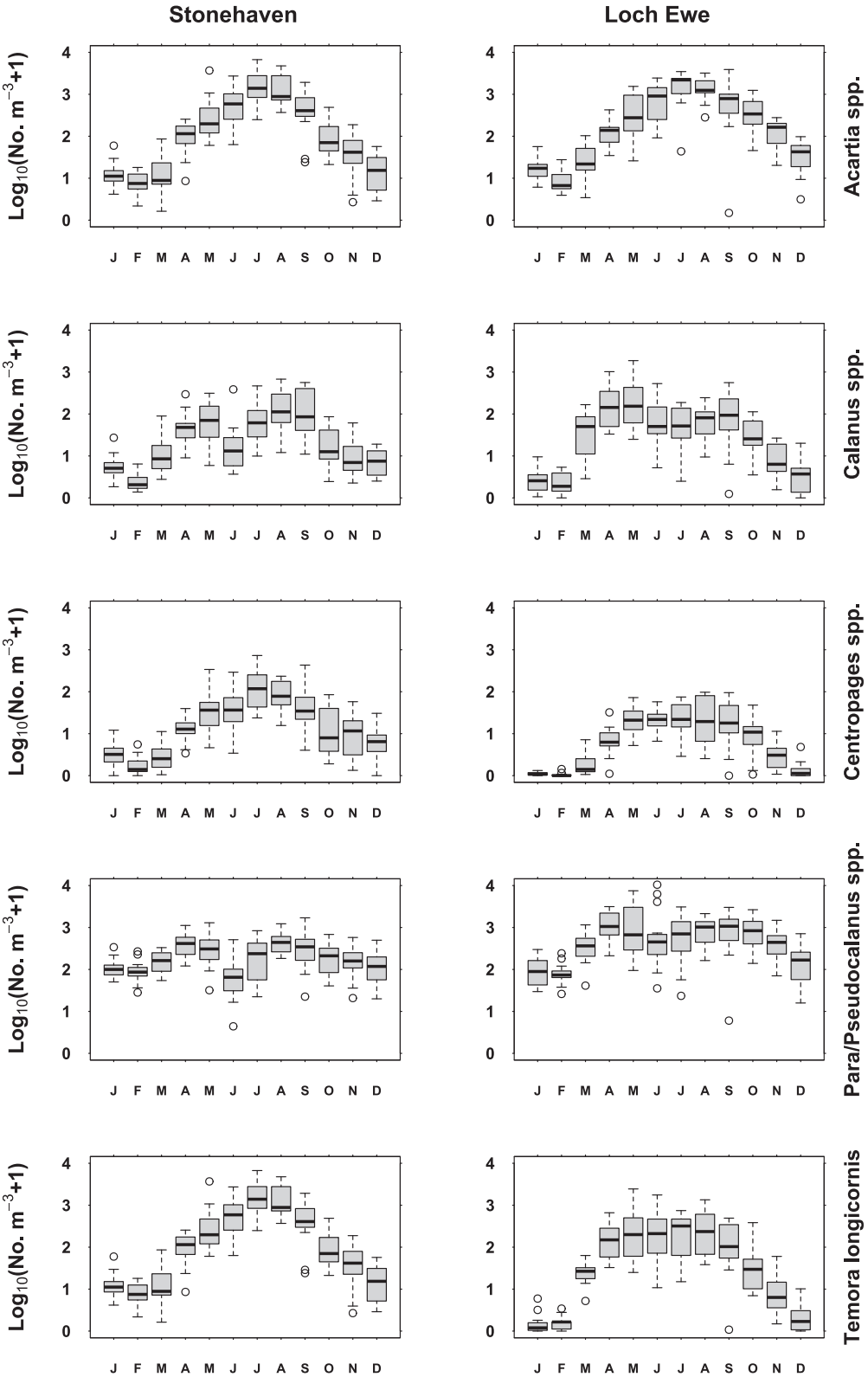

Figure 3. Box whisker plots of the monthly mean abundance of dominant copepod genera from Stonehaven and Loch Ewe 2002–2017.

respectively. The copepod genera had similar seasonal cycles on the east and west coast of Scotland during 2002–2017 (Figure 3). At both sites, all the common copepods are likely to encounter potential toxin producing *Pseudo-nitzschia* cells. All of the common copepods were present in high numbers during the autumn bloom when toxin producing species of *Pseudo-nitzschia* are more likely to be present (Fehling *et al.*, 2006). As a result the copepod genera detailed here were selected for the DA exposure experiment.

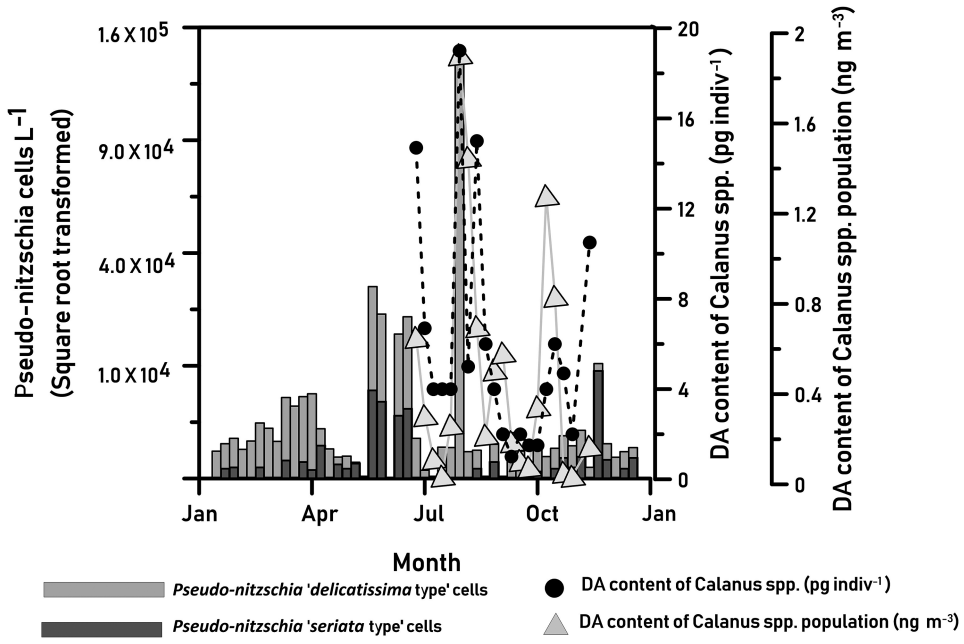

Figure 4. Seasonality of *Pseudo-nitzschia* "*delicatissima* type," "*seriata* type" cells, and DA content of *Calanus* individuals and population at Stonehaven during the field study period. *Pseudo-nitzschia* abundances are square root transformed and *y*-axis is labelled with untransformed values.

Pseudo-nitzschia spp. at the Stonehaven monitoring site in 2008

DA was analysed in field copepods at Stonehaven during 2008. *Pseudo-nitzschia* cells began to increase in abundance in February 2008 although numbers remained low, peaking at 4000 cells L^{-1} on March 12th (Supplementary Figure S1), dominated by *Pseudo-nitzschia* "*delicatissima* type" cells. *Pseudo-nitzschia* cells were numerically only a small component of the diatom bloom between mid May—mid June, reaching a cell density of ∼10000 cells L^{-1} with the community comprised of equal numbers of *Pseudo-nitzschia "delicatissima* type" and "*seriata* type" cells. A very short lived bloom of *Pseudo-nitzschia* comprised entirely of *P.* "*delicatissima* type" cells was recorded on the 22nd of July. This bloom was only observed during one sampling event (134880 cells L^{-1}) with low cell densities recorded the following week. These *Pseudo-nitzschia* "*delicatissima* type" cells were the main component of the diatom community at this time. *Pseudo-nitzschia* cell densities remained < 5000 cells L^{-1} until November when 9000 *P. "seriata* type" cells L^{-1} was recorded during one sampling event. Diatom cell densities then remained low during the rest of the year.

Analysis of plankton samples using TEM showed that the diversity of *Pseudo-nitzschia* at Stonehaven differed throughout 2008. On June 9th the *Pseudo-nitzschia* population was dominated by *P. seriata* cells with *Pseudo-nitzschia pungens* (Grunow ex Cleve) G. R. Hasle 1993 comprising a minor component of the population. On September 2nd, the *Pseudo-nitzschia* population comprised of *P. pungens* and *Pseudo-nitzschia fraudulenta* (Cleve) Hasle, 1993 in roughly equal proportions.This is the first record of *P. fraudulenta* from the Stonehaven monitoring site. On July 22nd the *Pseudo-nitzschia* bloom was dominated by a species belonging to the *Pseudo-nitzschia pseudodelicatissima* complex. These cells were similar in morphology to *Pseudo-nitzschia plurisecta* (called *P.* cf. *plurisecta* here) with 22–25 fibula and 39–44 striae in 10 μm, a central interspace and one row of poriods with 5–6 per 1 μm. The poroids where divided into multiple (between 4 and 10) sectors close to the edge of the hymen which varied in size. In some instances a central sector could be seen in the centre of the poroid, however unlike *Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle 2003 these central sectors were present in a minority of poroids. Cell width ranged from 1.2 to 1.5 μm, differing from measurements from Spain; 1.5–2.0 μm (Orive *et al.*, 2013), Namibia 1.5–1.8 μm (Gai *et al.*, 2018), and French Atlantic waters 1.9–2.5 μm (Caruana *et al.*, 2019). TEM images of *Pseudo-nitzschia* species from this analysis and morphological details of *P.* cf. *plurisecta* are given in Supplementary Figure S1 and Supplementary Table S3.

DA toxicity in field copepods

Calanus spp. at Stonehaven were found to already contain DA when analysis began in June 2008 with copepods containing 14.7 ρg DA $indiv^{-1}$ (Figure 4). DA content per individual subsequently declined, increasing in July to 19 ρg DA $indiv^{-1}$. This was the highest DA level per *Calanus* individual recorded in the study and coincident with the peak of *P.* cf. *plurisecta*. Following a week of lower DA toxicity, DA increased before declining again. DA concentrations remained low increasing to 6 ρg DA $indiv^{-1}$ in October. In November, DA toxicity per individual increased to 10 ρg DA $indiv^{-1}$ associated with an increase of *P. seriata* type cells to 9000 cells L^{-1}.

The relatively high DA content in *Calanus* individuals recorded at the beginning of the DA field study is not reflected in high DA levels in the *Calanus* population as *Calanus* spp. abundance was low. The highest number of total copepods during the DA field study was recorded at Stonehaven on this date but *Calanus* represented only 0.4% of the copepod population (Supplementary Figure S3). The highest DA levels in the *Calanus* population were recorded in July/August associated with the *P.* cf. *plurisecta* bloom (1.9 ng DA m^{-3}, Figure 4). High DA levels in the *Calanus* spp. popula-

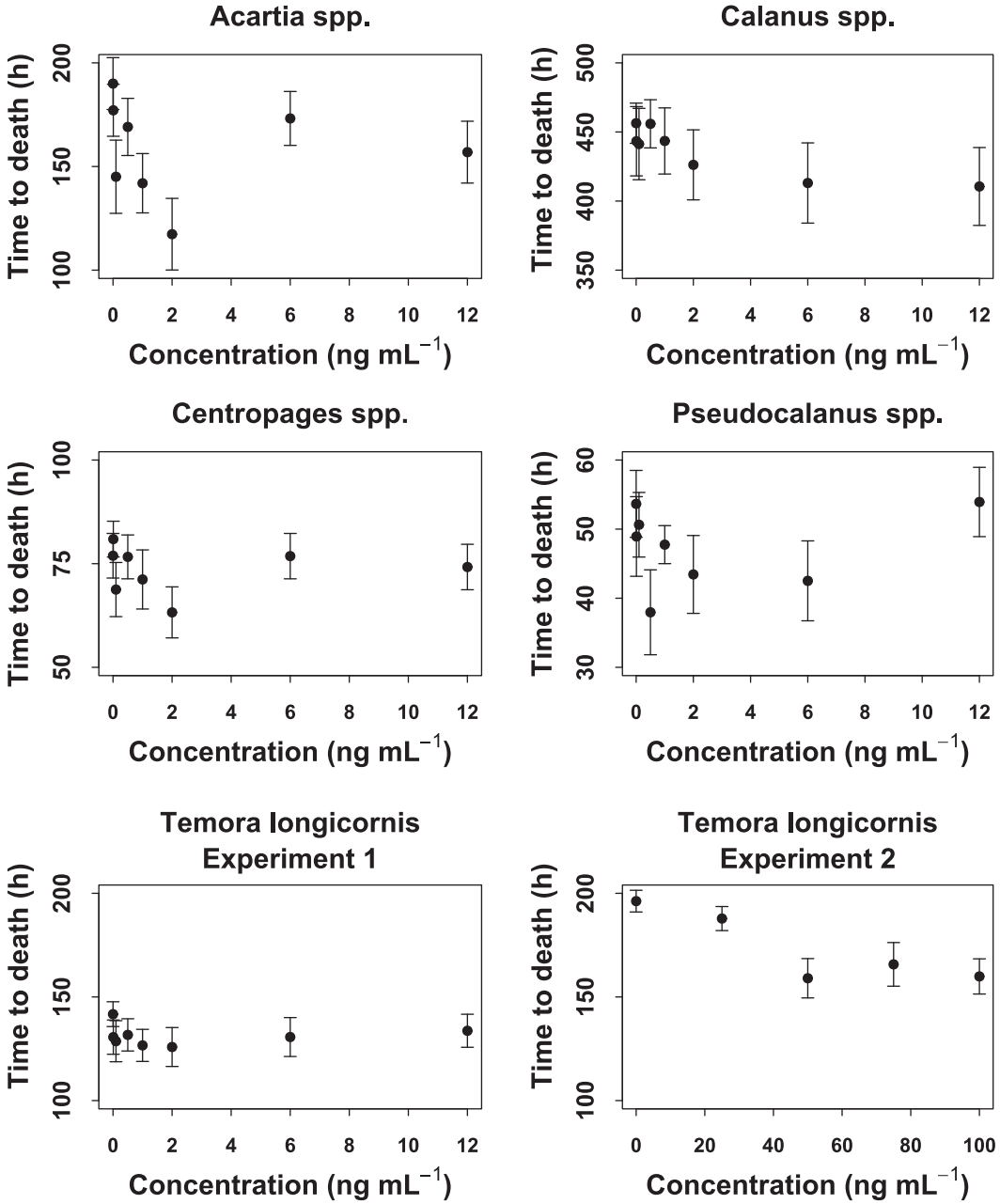

Figure 5. Average time to death (hours) in copepods exposed to dissolved DA, okadaic acid and saxitoxin. Vertical bars are standard errors. Note the different scales on the axes.

tion were also recorded in late September (1.3 ng m^{-3}) when the abundance of *Pseudo-nitzschia* was low. It should be noted that, although there was high toxicity of individual *Calanus* spp. in July, numerically they represented only 3% of the total copepod population at the time (Supplementary Figure S3). Even accounting for the larger *Calanus* body size, it remains likely that the toxin burden of the whole copepod community throughout this field DA study is higher.

DA exposure bioassay

There was no evidence of an effect on the swimming activity of copepods with any concentration of DA used in this study. Animals that were lethargic at one inspection had generally died before the next. Linear regressions showed no change in lethargy with increased concentration of DA for any genera of copepod (data not shown).

An LC_{50} was not achieved for any copepod species, even at the highest concentrations. *Calanus* spp. consistently took the most time to die, and *Pseudocalanus* spp. generally took the least time to die (Figure 5). Survival was significantly higher in experiment 2 compared to experiment 1 for *T. longicornis* incubations despite the higher toxin concentrations. Shared frailty Cox proportional-hazards regressions of time to death against toxin concentration was only statistically significant for *T. longicornis* (Table 1), although the coefficient of change in hazard was only 1.01 (i.e. for each 1 ng mL^{-1}

Table 1. Summary of Cox proportional-hazards regression with right censoring between time to death (hours) and DA concentration (ng mL^{-1}) for each species, with experiment number as an extra factor for *T. longicornis*. Results for all other copepod species are from experiment 1 only. Bold = significant at the 5% level.

	Variable	Proportion dead at end of experiment	*p*	% change risk of death with unit increase in variable (SE)
Acartia spp.	Concentration	0.73	0.93	No effect
Calanus spp.	Concentration	0.46	0.13	No effect
Centropages spp.	Concentration	0.56	0.45	No effect
Pseudocalanus spp.	Concentration	0.63	0.61	No effect
T. longicornis	Concentration	0.65	**0.00**	1.01 (0.003)
	Concentration and experiment number		**0.00**	

increase in DA the daily risk of *T. longicornis* death increased by 1%). The probability of survival after exposure to 50 ($p < 0.05$), 75 ($p < 0.1$), and 100 ng DA mL^{-1} ($p < 0.01$) was significantly lower than that of the control, although still higher than the control in experiment 1. *Temora longicornis* continued to feed on *P. micans*, with no mortality, when incubated with dissolved DA (10 ng mL^{-1}) for 24 h (mean ingestion ($\pm$ SE) = 44.6 ($\pm$ 11.0) cells $indiv^{-1}$ h^{-1}) but there was no detectable DA accumulated in the tissue.

Discussion

This study presents the first information on the potential exposure of copepods to toxin producing *Pseudo-nitzschia* in Scottish waters and provides evidence that copepods can act as a vector of DA to higher trophic levels. It also investigates the impact of dissolved DA on copepods in the Northeast Atlantic for the first time.

Seasonality of plankton

Examination of field data from two monitoring sites in Scotland show that copepods in this region are regularly exposed to potentially toxin producing *Pseudo-nitzschia* spp. during the phytoplankton growing period. Previous studies have shown copepod populations can develop resistance to algal toxins in locations where the two have historically co-existed (Dam, 2013). Differences in the abundance and seasonality of the potentially toxin producing *Pseudo-nitzschia* spp. can be observed between the two monitoring sites included in the study. The diversity of the *Pseudo-nitzschia* populations in Loch Ewe and Stonehaven have already been characterized with *Pseudo-nitzschia australis* Frenguelli 1939, *P. delicatissima* (Cleve) Heiden, 1928, *P. fraudulenta, P. pungens*, *P.* cf. *pseudodelicatissima* (Hasle) Hasle 1993, and *P. seriata* recorded in Loch Ewe (Bresnan *et al.*, 2016) and *P. australis, P. pungens, P. seriata*, and *P. subpacifica* (Hasle) Hasle, 1993 recorded in Stonehaven (Bresnan *et al.*, 2015). Fehling *et al.* (2004) confirmed *P. australis* and *P. seriata* as DA producers in Scottish waters. This study presents the first record of *P. fraudulenta* at the Stonehaven site and also the first record of *P.* cf. *plurisecta* in Scottish waters. The cell width of *P.* cf. *plurisecta* from Stonehaven measured here was less than that reported in previous studies (Orive *et al.*, 2013; Gai *et al.*, 2018; Caruana *et al.*, 2019), however these published measurements come from cultured cells and not from field material. Caruana *et al.* (2019) also confirmed *P. plurisecta* as a DA producer from French Atlantic waters. A short bloom of *P.* cf. *plurisecta* at Stonehaven was coincident with elevated DA concentrations in copepods suggesting that this species is a potential DA producer in Scottish waters.

Plankton dynamics during field study period

During the field study period, with the exception of July 22nd, *Pseudo-nitzschia* cell densities were relatively low. The trigger level for *Pseudo-nitzschia* cells in the Food Standards Scotland Shellfish Hygiene Monitoring Programme for the EU Shellfish Hygiene Directive (EU 2019/627) in Scotland is 50000 cells L^{-1} (S. Swan, pers. comm.). This threshold was only exceeded during the *P.* cf. *plurisecta* bloom in July. DA was detected in *Calanus* spp. during every sampling event and levels exceeded > 10 ρg $indiv^{-1}$ when cell densities of *Pseudo-nitzschia* were only ~ 9000 cells L^{-1}. This suggests that the *Calanus* community can accumulate DA, and potentially transfer it up the food chain, when *Pseudo-nitzschia* cell densities are less than those considered harmful in shellfish toxin monitoring programmes. Hall and Frame (2010) and Jensen *et al.* (2015) have reported the presence of DA in the faeces, urine, and amniotic fluid in seals in Scottish waters suggesting an active mechanism exists in this region where toxins can be vectored to top marine predators.

The low *Pseudo-nitzschia* cell densities of ~ 10000 cells L^{-1} during May/June occurred when copepods were most abundant. Whether these low *Pseudo-nitzschia* cell densities are a reflection of direct grazing by the copepod community on *Pseudo-nitzschia* cannot be ascertained, although the accumulation of DA in *Calanus* individuals during this period would suggest that grazing had taken place. DA production in *P. seriata* has been found to increase in the presence of copepods due to production of copepodamides by grazer species (Lundholm *et al.*, 2018; Harðardóttir *et al.*, 2019). Seasonal studies on the Swedish west coast have found copepodamides in the water column at levels (40 fM to 2 ρM) sufficient to induce toxin production response in diatoms (Selander *et al.*, 2019). A recent study by Trapp *et al.* (2021) found that the prediction of toxin levels in shellfish was improved by including copepod biomass and copepodamide concentration of shellfish extracts in the models. Thus, the potential exists that copepodamides may also be stimulating DA production in *P. seriata* during the elevated copepod abundances at Stonehaven. However, it is not possible to ascertain if the resultant toxicity in *Calanus* spp. in this study was as a direct result of copepodamide driven DA production by *P. seriata*.

DA impacts on copepods

The impacts of DA on copepods is varied. Consumption of DA producing *Pseudo-nitzschia* (reviewed by Bates *et al.*, 2018) can have a harmful effect on the behaviour and mortality of zooplankton grazers although some laboratory studies investigating effects of ingested DA have found little effect on copepod grazing or reproduction (Lincoln *et al.*, 2001; Maneiro *et al.*, 2005; Harðardóttir *et*

al., 2015; Miesner *et al.*, 2016). However, Harðardóttir *et al.* (2018) found that *Calanus hyperboreus* feeding on toxin producing *P. seriata* had reduced escape response within 12 h, but impacts were only observed in *C. glacialis* after 72 h. The impact of consuming toxin producing *Pseudo-nitzschia* or accumulation of DA on the escape response of copepods in Scottish waters is still unknown.

Impacts from exposure to algal toxins via active excretion from algal cells, passive leakage from senescent cells, or cell lysis can include regurgitation, rapid heartbeat, loss of motor control, incapacitation, and change in swimming behaviour (e.g. Sopanen *et al.*, 2011; Hong *et al.*, 2012; Lasley-Rasher *et al.*, 2016; Harðardóttir *et al.*, 2018). In this study, there was no obvious physiological effect of toxin concentration on swimming or feeding activity from exposure to dissolved DA. In most cases, if an animal was recorded as a lethargic swimmer during one observation, it had died by the next day regardless of toxin concentration, suggesting that the lethargy was due to poor physiological condition caused by starvation rather than due to the action of toxins.

The results presented here suggest that dissolved DA does not have a significant effect on mortality of *Acartia* spp., *Calanus* spp., *Centropages* spp., or *Pseudocalanus* spp. There was some evidence that suggested high levels of DA ($\geq$ 50 ng mL^{-1}) did decrease survival of *T. longicornis* by a small amount (1% increase in risk of death with a 1 ng mL-1 increase in DA). The experimental concentrations used in this experiment are low compared to levels found in the North Pacific, where 136 ng mL^{-1} have been recorded in the field (Trainer *et al.*, 2007) and to LC_{50}s from previous experimental studies (2.86, 37.5, and 135 µg DA mL^{-1} for *T. californicus* from the Pacific, *Pseudocalanus acuspes*, and *T. longicornis* from Nova Scotia, respectively; Shaw *et al.*, 1997; Windust, 1997). However, this experiment was designed to test concentrations that would represent a "worst case" scenario for Scottish waters given the information from the previous 6 years of monitoring data. Toxin contents of *Pseudo-nitzschia* cells in Scottish waters of up to 0.23 pg cell^{-1} (Fehling *et al.*, 2004) have been measured, which would equate to a dissolved DA concentration of 391 ng mL^{-1} if all the *Pseudo-nitzschia* spp. cells in the water column, at the highest concentration observed at Stonehaven to date (1.7×10^6 cells L^{-1}, Bresnan *et al.*, 2015), belonged to a toxin producing species and expelled their toxin contents. Studies have shown that the extracellular fraction of DA in a culture may constitute up to 97% of the total DA under culture conditions (Godinho *et al.*, 2018), therefore, it is possible that effects of dissolved DA on the survival of *T. longicornis* could be seen in the wild at Stonehaven during bloom conditions that are higher than current biotoxin monitoring programme trigger levels. In reality, the amount of toxin released into the water column in the field will be lower than the total concentration of DA in *Pseudo-nitzschia* cells as DA undergoes photodegradation (Bouillon *et al.*, 2006) and has been shown to be a component of aggregates of *Pseudo-nitzschia* cells in marine snow sinking to the sea bed (Schnetzer *et al.*, 2017).

Copepods as a vector of DA to higher trophic levels

Copepods are an important food source for fish larvae and pelagic fish such as mackerel and herring (e.g. Prokopchuk and Sentyabov, 2006; Falkenhaug and Dalpadado, 2013; Jansen, 2016). Although fish are not directly affected by DA ingestion (Lefebvre *et al.*, 2012) they can accumulate DA to levels that are toxic to higher trophic levels. Results from this study show that *Calanus* spp. can act as a source of DA for higher levels of the food chain. The highest toxicity during this study was observed in the *Calanus* spp. population after a bloom of *P.* cf. *plurisecta*. Kershaw *et al.* (2021) has shown DA contamination of a variety of different fish from different regions in Scotland. At a whole fish level, highest DA concentrations were found in pelagic species (mostly mackerel and herring), gadoids, and flatfish species. Highest concentrations of DA were found in fish from the east coast of Scotland and DA was found in fish over 8 months suggesting a relatively constant exposure to DA. *Calanus* tested positive for DA throughout the study period. At the Stonehaven monitoring site *Pseudo-nitzschia* is recorded in nearly every weekly phytoplankton sample since monitoring began, although sometimes close to the limit of detection (20 cells L^{-1}; Bresnan *et al.*, 2015) suggesting the potential for constant exposure to DA even at very low levels. Bresnan *et al.* (2015) has shown interannual variability in the timing of high *Pseudo-nitzschia* abundances, which have been recorded in spring, summer or autumn in different years and thus the timing of exposure of zooplankton to DA may vary from year to year as the phenology and, potentially, composition of the *Pseudo-nitzschia* populations change. *Calanus* spp. represented a minor component of the copepod community abundance during the study period, comprising from < 1–15% of the total copepod abundance. Therefore, even considering the larger body size of *Calanus* spp. compared to the rest of the copepods at Stonehaven, the level of DA in the total copepod community is likely to be higher than that measured during this study.

Conclusions

Calanus spp. have been confirmed as potential vectors of DA to higher trophic levels in Scottish waters. Accumulation of DA toxins in *Calanus* spp. at levels > 10 ρg indiv^{-1} was associated with *Pseudo-nitzschia* cell densities ranging from ∼9000 to 135000 cells L^{-1}. The accumulation of DA in *Calanus* individuals at low *Pseudo-nitzschia* cell densities suggests that at certain times of year phytoplankton trigger levels used in shellfish monitoring programmes will not flag potential for DA accumulation in copepods.

The highest DA concentrations tested ($\geq$ 50 ng DA mL^{-1}) decreased survival slightly in *T. longicornis* but did not impact other members of the copepod community. The results from this study provide evidence that while copepods can vector DA up the food chain, they will not be impacted by the presence of dissolved DA in the water column.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Authors contributions

KC and EB conceived of and designed the study. KC coordinated the study. KC and JL carried out the experiments. EB and MM provided time-series data. KC and EB drafted the manuscript. All authors read and approved the final manuscript.

Funding

The monitoring programme is performed as part of the Scottish Government Service Level Agreement (ST05a). Dissolved toxin experiments were performed under Fisheries Research Services ROAME AE1198.

Availability of data and materials

Monitoring data are available from https://data.marine.gov.scot/group/monitoring, experimental data is available as supplementary material.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors acknowledge the contribution of Mari Ann Maskew, Shona Kinnear, Keir Hardy, and Elizabeth Turrell for their input to this study. The authors are grateful to the sampling team and crew of the R.V. Temora for collecting plankton samples and seawater from Stonehaven, Phil MacLachlan, Jane, and Willie Grant for collecting plankton samples from Loch Ewe, and all plankton analysts involved in the Scottish Coastal Observatory. The authors also wish to thank the two anonymous reviewers whose comments have greatly improved this manuscript.

This manuscript is dedicated to the memory of Steve Hay who played an integral role in zooplankton taxonomy and ecology science in MSS as well as the establishment of SCObs over the course of his career.

References

Bates, S. S., Hubbard, K. A., Lundholm, N., Montresor, M., and Leaw, C. P. 2018. *Pseudo-nitzschia*, *nitzschia*, and domoic acid: new research since 2011. Harmful Algae, 79: 3–43.

Bedford, J., Ostle, C., Johns, D. G., Atkinson, A., Best, M., Bresnan, E., Machairopoulou, M. *et al.* 2020. Lifeform indicators reveal large-scale shifts in plankton across the North-West European shelf. Global Change Biology, 26: 3482–3497.

Bouillon, R.-C., Knierim, T. L., Kieber, R. J., Skrabal, S. A., and Wright, J. L. C. 2006. Photodegradation of the algal toxin domoic acid in natural water matrices. Limnology and Oceanography, 51: 321–330.

Bresnan, E., Arévalo, F., Belin, C., Branco, M. A. C., Cembella, A. D., Clarke, D., Correa, J. *et al.* 2021. Diversity and regional distribution of harmful algal events along the Atlantic margin of Europe. Harmful Algae, 102: 101976.

Bresnan, E., Cook, K., Hindson, J., Hughes, S., Lacaze, J.-P., Walsham, P., Webster, L. *et al.* 2016. The Scottish coastal observatory 1997-2013 part 2 – description of Scotland's coastal waters. Scottish Marine and Freshwater Science, 7: 278.

Bresnan, E., Fryer, R. J., Fraser, S., Smith, N., Stobo, L., Brown, N., and Turrell, E. 2017. The relationship between *Pseudo-nitzschia* (Peragallo) and domoic acid in Scottish shellfish. Harmful Algae, 63: 193–202.

Bresnan, E., Kraberg, A., Fraser, S., Brown, L., Hughes, S., and Wiltshire, K. H. 2015. Diversity and seasonality of *Pseudo-nitzschia* (Peragallo) at two North Sea time-series monitoring sites. Helgoland Marine Research, 69: 193–204.

Brown, R. J., Rundle, S. D., Hutchinson, T. H., Williams, T. D., and Jones, M. B. 2005. A microplate freshwater copepod bioassay for evaluating acute and chronic effects of chemicals. Environmental Toxicology and Chemistry, 24: 1528–1531.

Caruana, A. M. N., Ayache, N., Raimbault, V., Rétho, M., Hervé, F., Bilien, G., Amzil, Z. *et al.* 2019. Direct evidence for toxin production by *Pseudo-nitzschia plurisecta* (Bacillariophyceae) and extension of its distribution area. European Journal of Phycology, 54: 585–594.

Dam, H. G. 2013. Evolutionary adaptation of marine zooplankton to global change. Annual Review of Marine Science, 5: 349–370.

Dam, H. G., and Lopes, R. M. 2003. Omnivory in the calanoid copepod *Temora longicornis*: feeding, egg production and egg hatching rates. Journal of Experimental Marine Biology and Ecology, 292: 119–137.

Falkenhaug, T., and Dalpadado, P. 2013. Diet composition and food selectivity of sprat (*Sprattus sprattus*) in Hardangerfjord, Norway. Marine Biology Research, 10: 203–215.

Fehling, J., Davidson, K., Bolch, C., and Tett, P. 2006. Seasonality of *Pseudo-nitzschia* spp. (Bacillariophyceae) in western Scottish waters. Marine Ecology Progress Series, 323: 91–105.

Fehling, J., Green, D. H., Davidson, K., Bolch, C. J., and Bates, S. S. 2004. Domoic acid production by *Pseudo-nitzschia seriata* (Bacillariophyceae) in Scottish waters. Journal of Phycology, 40: 622–630.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepods *Calanus pacificus*. Limnology and Oceanography, 17: 805–815.

Gai, F. F., Hedemand, C. K., Louw, D. C., Grobler, K., Krock, B., Moestrup, Ø., and Lundholm, N. 2018. Morphological, molecular and toxigenic characteristics of namibian *Pseudo-nitzschia* species – including *Pseudo-nitzschia bucculenta* sp. nov. Harmful Algae, 76: 80–95.

Gallacher, S., Howard, F. G., Hess, P., Macdonald, E. M., Kelly, M. C., Bates, L. A., Brown, N. *et al.* 2001. The occurrence of amnesic shellfish poisons in shellfish from Scottish waters. *In* Harmful Algal Blooms 2000, pp. 30–33. Ed. by Hallegraeff, G. M, Blackburn, S. I., Bolch, C. J., and Lewis, R. J.. Intergovernmental Oceanographic Commission of UNESCO, Paris.

Godinho, L., Silva, A., Branco, Castelo, M., A., Marques, A., and Costa, P. R. 2018. Evaluation of intracellular and extracellular domoic acid content in *Pseudo-nitzschia multiseries* cell cultures under different light regimes. Toxicon, 155: 27–31.

Hall, A. J., and Frame, E. 2010. Evidence of domoic acid exposure in harbour seals from Scotland: a potential factor in the decline in abundance? Harmful Algae, 9: 489–493.

Harðardóttir, S., Hjort, D. M., Wohlrab, S., Krock, B., John, U., Nielsen, T. G., and Lundholm, N. 2019. Trophic interactions, toxicokinetics, and detoxification processes in a domoic acid-producing diatom and two copepod species. Limnology and Oceanography, 64: 833–848.

Harðardóttir, S., Krock, B., Wohlrab, S., John, U., Nielsen, T. G., and Lundholm, N. 2018. Can domoic acid affect escape response in copepods? Harmful Algae, 79: 50–52.

Harðardóttir, S., Pančić, M., Tammilehto, A., Krock, B., Møller, E., Nielsen, T., and Lundholm, N. 2015. Dangerous relations in the Arctic marine food web: interactions between toxin producing *Pseudo-nitzschia* diatoms and *Calanus* copepodites. Marine Drugs, 13: 3809.

Hong, J., Talapatra, S., Katz, J., Tester, P. A., Waggett, R. J., and Place, A. R. 2012. Algal toxins alter copepod feeding behavior. Plos ONE, 7: e36845.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs, 54: 187–211.

Jansen, T. 2016. First-year survival of North East Atlantic mackerel (*Scomber scombrus*) from 1998 to 2012 appears to be driven by availability of *calanus*, a preferred copepod prey. Fisheries Oceanography, 25: 457–469.

Jensen, S.-K., Lacaze, J.-P., Hermann, G., Kershaw, J., Brownlow, A., Turner, A., and Hall, A. 2015. Detection and effects of harmful algal toxins in Scottish harbour seals and potential links to population decline. Toxicon, 97: 1–14.

Karlson, B., Andersen, P., Arneborg, L., Cembella, A., Eikrem, W., John, U., West, J. J. *et al.* 2021. Harmful algal blooms and their effects in coastal seas of northern Europe. Harmful Algae, 102: 101989.

Kershaw, J. L., Jensen, S.-K., McConnell, B., Fraser, S., Cummings, C., Lacaze, J.-P., Hermann, G. *et al.* 2021. Toxins from harmful algae in fish from Scottish coastal waters. Harmful Algae, 105: 102068.

Kiørboe, T., Saiz, E., Tiselius, P., and Andersen, K. H. 2018. Adaptive feeding behavior and functional responses in zooplankton. Limnology and Oceanography, 63: 308–321.

Lasley-Rasher, R. S., Nagel, K., Angra, A., and Yen, J. 2016. Intoxicated copepods: ingesting toxic phytoplankton leads to risky behaviour. Proceedings of the Royal Society B Biological Sciences, 283: 20160176.

Lefebvre, K. A., Frame, E. R., and Kendrick, P. S. 2012. Domoic acid and fish behavior: a review. Harmful Algae, 13: 126–130.

Lelong, A., Hégaret, H., Soudant, P., and Bates, S. S. 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. Phycologia, 51: 168–216.

Lincoln, J. A., Turner, J. T., Bates, S. S., Léger, C., and Gauthier, D. A. 2001. Feeding, egg production, and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets of the toxic diatom *Pseudo-nitzschia multiseries* and the non-toxic diatom *Pseudo-nitzschia pungens*. Hydrobiologia, 453/454: 107–120.

Lundholm, N., Hansen, P. J., and Kotaki, Y. 2005. Lack of allelopathic effects of the domoic acid-producing marine diatom *Pseudo-nitzschia multiseries*. Marine Ecology Progress Series, 288: 21–33.

Lundholm, N., Hasle, G. R., Fryxell, G. A., and Hargraves, P. E. 2002. Morphology, phylogeny and taxonomy of species within the *Pseudo-nitzschia americana* complex (Bacillariophyceae) with descriptions of two new species, *Pseudo-nitzschia brasiliana* and *Pseudo-nitzschia linea*. Phycologia, 41: 480–497.

Lundholm, N., Krock, B., John, U., Skov, J., Cheng, J., Pančić, M., Wohlrab, S. *et al.* 2018. Induction of domoic acid production in diatoms—types of grazers and diatoms are important. Harmful Algae, 79: 64–73.

Lundholm, N., Moestrup, Ø., Hasle, G. R., and Hoef-Emden, K. 2003. A study of the *Pseudo-nitzschia pseudodelicatissima/cuspidata* complex (Bacillariophyceae): what is *P . pseudodelicatissima*? Journal of Phycology, 39: 797–813.

Lundholm, N., Moestrup, Ø., Kotaki, Y., Hoef-Emden, K., Scholin, C., and Miller, P. 2006. Inter- and intraspecific variation of the *Pseudo-nitzschia delicatissima* complex (Bacillariophyceae) illustrated by rRNA probes, morphological data and phylogenetic analyses. Journal of Phycology, 42: 464–481.

Maneiro, I., Iglesias, P., Guisande, C., Riveiro, I., Barreiro, A., Zervoudaki, S., and Granéli, E. 2005. Fate of domoic acid ingested by the copepod *Acartia clausi*. Marine Biology, 148: 123–130.

Mann, K. H. 1993. Physical oceanography, food chains, and fish stocks: a review. ICES Journal of Marine Science, 50: 105–119.

Miesner, A. K., Lundholm, N., Krock, B., and Nielsen, T. G. 2016. The effect of *Pseudo-nitzschia seriata* on grazing and fecundity of *Calanus finmarchicus* and *Calanus glacialis*. Journal of Plankton Research, 38: 564–574.

Orive, E., Pérez-Aicua, L., David, H., García-Etxebarria, K., Laza-Martínez, A., Seoane, S., and Miguel, I. 2013. The genus *Pseudo-nitzschia* (Bacillariophyceae) in a temperate estuary with description of two new species: *Pseudo-nitzschia plurisecta* sp. nov. and *Pseudo-nitzschia abrensis* sp. nov. Journal of Phycology, 49: 1192–1206.

Prokopchuk, I., and Sentyabov, E. 2006. Diets of herring, mackerel, and blue whiting in the Norwegian Sea in relation to *Calanus finmarchicus* distribution and temperature conditions. ICES Journal of Marine Science, 63: 117–127.

Quilliam, M. A., Xie, M., and Hardstaff, W. R. 1995. Rapid extraction and cleanup for liquid chromatographic determination of domoic acid in unsalted seafood. Journal of AOAC International, 78: 543–554.

Régnier, T., Gibb, F. M., and Wright, P. J. 2017. Importance of trophic mismatch in a winter- hatching species: evidence from lesser sandeel. Marine Ecology Progress Series, 567: 185–197.

Rondeau, V., Marzroui, Y., and Gonzalez, J. R. 2012. frailtypack: an r package for the analysis of correlated survival data with frailty models using penalized likelihood estimation or parametrical estimation. Journal of Statistical Software, 47: 1–28.

Runge, J. A. 1988. Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. Hydrobiologia, 167-168: 61–71.

Schnetzer, A., Lampe, R. H., Benitez-Nelson, C. R., Marchetti, A., Osburn, C. L., and Tatters, A. O. 2017. Marine snow formation by the toxin-producing diatom, *Pseudo-nitzschia australis*. Harmful Algae, 61: 23–30.

Selander, E., Berglund, E. C., Engström, P., Berggren, F., Eklund, J., Harðardóttir, S., Lundholm, N. *et al.* 2019. Copepods drive large-scale trait-mediated effects in marine plankton. Science Advances, 5: eaat5096.

Selander, E., Kubanek, J., Hamberg, M., Andersson, M. X., Cervin, G., and Pavia, H. 2015. Predator lipids induce paralytic shellfish toxins in bloom-forming algae. Proceedings of the National Academy of Sciences, 112: 6395–6400.

Shaw, B. A., Andersen, R. J., and Harrison, P. J. 1997. Feeding deterrent and toxicity effects of apo-fucoxanthinoids and phycotoxins on a marine copepod (*Tigriopus californicus*). Marine Biology, 128: 273–280.

Skov, J., Lundholm, N., Moestrup, Ø., and Larsen, J. 1999. Potentially toxic phytoplankton. 4. The diatom genus *Pseudo-nitzschia* (Diatomophyceae/Bacillariophyceae). ICES identification leaflets for phytoplankton, 185: 1–23.

Smith, L. E. 2018. Climate Change Effects on Copepod Physiology and Trophic Transfer. p 53. University of Delaware.

Sopanen, S., Setälä, O., Piiparinen, J., Erler, K., and Kremp, A. 2011. The toxic dinoflagellate *Alexandr ium ostenfeldii* promotes incapacitation of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa* from the northern Baltic Sea. Journal of Plankton Research, 33: 1564–1573.

Thessen, A. E., and Stoecker, D. K. 2008. Distribution, abundance and domoic acid analysis of the toxic diatom genus *Pseudo-nitzschia* from the Chesapeake Bay. Estuaries and Coasts, 31: 664–672.

Throndsen, J. 1978. Preservation and storage. *In* Phytoplankton Manual, pp. 69–74. Ed. by Sournia, A.. UNESCO, Paris.

Trainer, V. L., Cochlan, W. P., Erickson, A., Bill, B. D., Cox, F. H., Borchert, J. A., and Lefebvre, K. A. 2007. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. Harmful Algae, 6: 449–459.

Trapp, A., Heuschele, J., and Selander, E. 2021. Eavesdropping on plankton—can zooplankton monitoring improve forecasting of biotoxins from harmful algae blooms? Limnology and Oceanography, 66: 3455–3471.

Turner, J. T. 2014. Planktonic marine copepods and harmful algae. Harmful Algae, 32: 81–93.

Utermöhl, H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitteilungen Internationale Vereinigung für Theoretische und Angewandte Limnologie, 9: 1–38.

Wells, S. R., Bresnan, E., Cook, K., Eerkes-Medrano, D., Machairopoulou, M., Mayor, D. J., Rabe, B. *et al.* in press. Environmental drivers of a decline in a coastal zooplankton community. ICES Journal of Marine Science, https://doi.org/10.1093/icesjms/fsab177.

Willis, K. J., and Ling, N. 2004. Toxicity of the aquaculture pesticide cypermethrin to planktonic marine copepods. Aquaculture Research, 35: 263–270.

Windust, A. 1997. The Responses of Bacteria, Microalgae, and Zooplankton to the Diatom Nitzschia pungens f. multiseries and its Toxic Metabolite Domoic Acid. p. 107p. Dalhousie University, Halifax, Canada.

Handling Editor: Juan Bellas