Testing For A Relationship Between The Tidal Cycle And Abundance Of *Corophium Volutator* (Pallas, 1766) (Amphipoda, Corophiidae)

In The Stour Estuary (Kent, Uk)

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TESTING FOR A RELATIONSHIP BETWEEN THE TIDAL CYCLE AND ABUNDANCE OF <u>COROPHIUM VOLUTATOR</u> (PALLAS, 1766) (AMPHIPODA, COROPHIIDAE)

IN THE STOUR ESTUARY (KENT, UK)

BY

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ABSTRACT

The distribution of <u>Corophium volutator</u> has been investigated by several authors yet, to our knowledge very little is known about the relationship between the complete tidal cycle and their abundance. An investigation wastherefore conducted to identify the relationship between the abundance of <u>C. volutator</u> and the effect of the tidal cycle on the intertidal mudflats at Richborough Port. Salinity levels, weight of suspended solids, and immersion and exposure times, were measured during the ebb and flow of the complete tidal cycle. Sampling was conducted during the winter, which can be a time of high abiotic stress. Some protection was afforded from the tidal flow by the presence of an old jetty which enclosed the left hand side of the sampling area. However, results confirmed that there was no relationship between

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the abundance of <u>C.volutator</u> and any of the tidal variables measured. The absence of a relationship could suggest that other confounding variables may have been causing an effect on Corophid distribution and abundance.

INTRODUCTION

The burrowing Amphipod <u>C. volutator</u> is an abundant crustacean species found in the upper 5cm of sediment of many intertidal mudflats across Europe and America (Debacker et al. 2010 & Watkin, 1941). This small crustacean ranges in length from 1-11mm and can be found on the silty bank of the river Stour at Richborough Port. <u>C.volutator</u> feeds on suspended solids by filtering particles from the current, created by the hydrodynamics of the U shaped burrows (McLusky, 1968). Burrows are constructed by the females, which are co-inhabited by the males (Forbes et al. 2006). Feeding is performed by the beating of the pleopods and through the scraping of the antennae to direct suspended material into the current (Meadows & Reid, 1966). <u>C.volutator</u> is predominantly a deposit feeder, consuming benthic diatoms and bacterial films or sand particles suspended in the ambient water (Creach et al. 1997). For this experiment suspended solids contained within water samples taken at different times of the tidal cycle were measured to give an indication of the amount of available nutrients for <u>C.volutator</u>.

The swimming behaviour of <u>C.volutator</u> is influenced by semi-lunar and seasonal factors, mainly occurring during the summer period and at night during the ebb tide period (Hughes, 1988; De Backer et al. 2010; Morgan, 1965). For this reason Corophid sampling was conducted during the day when the animals have been reported to reside in their burrows (Hughes & Horsfall, 1990). Morgan (1965) suggested that tidal variations in hydrostatic pressure changes, rather than changes in water currents or mechanical stimulation by surface

waves, are responsible for the synchronisation of entrainment of swimming behavioural rhythms in <u>C.volutator</u> (Morgan, 1965).

These tube dwelling amphipods rarely leave their burrows except during the reproductive season when males will crawl on the sediment surface into different burrows to reproduce with females (Barbeau & Grecian, 2003; and Forbes et al. 2006). Females Corophids are receptive to mating for a few days after their moult whilst mating opportunities for males may vary over the tidal cycle and among different levels of the intertidal zone (McCurdy et al. 2000). These obstinate periods are the result of the hardening of the brood pouch restricting successful fertilisation and reduction of brood pheromones (Borowsky, 1991). <u>C.volutator</u> has two generations per year, a summer generation that reproduces until October and an over-winter generation that dies off over the summer (Fish & Mills, 1979). The winter generation of C.volutator were sampled for abundance on 21 January 2012.

Maximum densities of the species have been found to show a preference of silty substratum and the presence of organic material within fine grain sediments like the silty substratum of the Richborough mudflats (Anderson, 1972). Following the introduction of the invasive species <u>Chelicorophium curvispinum</u> to the River Stour, the population size of <u>C.volutator</u> has dramatically increased in the lower part of the Stour estuary since the summer of 1998 (Buckley et al. 2004). Large population densities make <u>C.volutator</u> an important keystone species in many mudflat ecosystems (Debacker et al. 2010). The high lipid content of this amphipod provides an important food source for migrating shorebirds such as the sandpiper (Hilton et al. 2002). However, few predatory birds have been observed at the sampling site.

Mouritsen et al (1998) found that this amphipod was predominantly essential in stabilizing sediments and maintaining the mosaic of emerged areas on a mudflat (Drolet & Barbeau, 2009). Distribution of <u>C.volutator</u> is frequently considered to be patchy at different

spatial extents (Drolet & Barbeau, 2009), often occurring below the neap tide mark of ordinary tides (Watkin, 1941). <u>C.volutator</u> populations that dominate soft substrate community often exhibit zonal distribution patterns along the tidal gradient (Kneib, 1984). Corophids found along the mudflats of the sampling site at Richborough Port support these findings, appearing in a linear band parallel to the shore. However, some individuals were observed in nearby tide pools. Conferring to Drolet & Barbeau (2009), tide pools surrounding the sampling site were not affected by physical stress as strongly as zones that are exposed at low tide. Shelter from the nearby Jetty may have influenced the abundance and distribution of <u>C.volutator</u> protecting the burrows from the main force of the tide.

A Previous study by Van den Huvel-Greve et al. (2007) suggests that the mortality, growth rate, and reproduction of <u>C.volutator</u> are affected by the salinity and oxygen saturation of the ambient water. During this study salinity measurements were taken at different times of the tidal cycle to investigate the influence of salinity on the abundance of <u>C.volutator</u>.

To our knowledge, very little is known about the relationship between the complete tidal cycle and the abundance of <u>C.volutator</u> in the field. This study aims to identify a relationship between the tidal cycle and the abundance of <u>C.volutator</u> located on the mudflats of the river Stour Estuary at Richborough Port. Variables within <u>C.volutator's</u> niche requirements such as the salinity, weight of suspended solids, tidal flow rate, exposure and immersion times, were measured during the progressive ebb and flow of the tide. The purpose of this experiment was to test the working hypothesis that a relationship exists between the abundance of <u>C.volutator</u> and the salinity, amount of suspended solids, flow rate, and exposure and immersion times of during the progressive ebb and flow of the tide (see Fig.1 for details).

Study Area

The River Stour is located in the south-east of England and instigates from two main tributaries, the East Stour and the Great Stour, emptying into Sandwich Bay. The study area was situated at Richborough Port on the intertidal mudflats on the south bank of the river Stour, near the mouth of the river, west of an old jetty. The river is subject to a semi-diurnal tide, predisposed by the hydrography of the river bed. The Salinity of the English Channel penetrates the river affecting the mudflats of Richborough Port located at the mouth of the river (Buckley et al. 2004). The river carried a high concentration of suspended solids and detritus ranging from 0.005g to 0.237g with salinity measurements ranging from 0.24 mg/l to 0.38 mg/l. The benthic substratum primarily consisted of fine sediment of sand and thick sediment.

MATREIAL AND METHODS

The experiment took place on 21 January 2012 during a neap tide. The study area encompassed the Corophid habitat, surrounding a band of distributed <u>C.volutator</u> burrows, parallel with the tide. Ten bamboo sticks were positioned 0.2m apart heading out towards the shore for 2m, perpendicular to the tide. The following 5m of bamboo sticks were positioned 1m apart. This was because of adaption of the experiment in the light of the conditions on the day of the experiment (see Fig. 2 for details).

Three replicates of sediment samples containing <u>C.volutator</u> were collected using cuboid shaped plastic containers measuring (L: 5.5cm × W: 4.2cm × H: 3.9cm). The three replicates were taken approximately 0.3m apart from each other parallel to the tide in line with each bamboo stick marker. Samples were taken by stamping the plastic containers into

the sediment, retracting them to attain a sediment sample equivalent to the depth of the container. Each sample was taken as the ebbing tide passed each bamboo stick marker. The containers containing the sediment and <u>C.volutator</u> samples were then sealed with water tight lids ready to be taken back to the laboratory for analysis.

15 clean 500ml plastic bottles were used to collect water samples of the River Stour labelled 1a-15a for the flowing tide and 1b-15b for the ebbing tide. These samples were collected during a complete tidal cycle as the ebb and flow of the tide passed each bamboo marker. Sampling was conducted from leaning over the nearby jetty to avoid disturbing the <u>C.volutator</u> in the sediment. Times of water sampling throughout the tidal cycle were recorded and immersion and exposure times of the <u>C.volutator</u> burrows were calculated. The speed of the ebbing tide was recorded as an adaption of the experiment in light of the windy conditions on the day of the experiment.

Back at the laboratory water samples were placed in a freezer for two days. Individual sediment samples were sieved for <u>C.volutator</u> using a circular 0.3mm mesh sieve and a bucket filled with water. The remaining filtrate containing <u>C.volutator</u> and detritus from the river were placed in clean containers containing 70% alcohol for biological preservation. These containers were re-sieved the following day using a smaller diameter 0.3mm mesh sieve. After the second filtration, the remaining filtrate containing Corophids from each of the sediment samples were individually sorted using a viewing tray containing 70% alcohol and placed under a dissecting microscope. The abundance of <u>C.volutator</u> contained in each sediment sample was identified. Each identified individual Corophid was counted and carefully extracted from the viewing tray using a pair of forceps to be placed in a labelled vial containing 70% alcohol for biological preservation. The average abundance of <u>C.volutator</u> was then calculated for each of the sampling bands, marked by the bamboo markers.

30 water samples were removed from the freezer two days after freezing and left to thaw out overnight. 500ml of each water sample was filtered through labelled filter paper which corresponded to each water sample. Each sheet of filter paper was weighed individually using scales accurate to one hundredth of a gram. Water samples were then filtered using the corresponding filter paper by the Buchner filtration method (Wakerman & Tarleton, 2005). After each filtration, filter paper containing the filtrate residue was placed in the oven at 60°C and left to dry overnight. The dried filtrate paper was re-weighed the following morning and the weight of the filtrate was measured to determine the weight of the suspended solids within the ambient water during the tidal cycle.

The filtered water samples were transferred to clean bottles in preparation for Sodium analysis using the Flame Photometer (Jenway manufacturers). To prepare the stock solution, 2.54g of Sodium Chloride was dissolved in 11 of distilled water. Four conical flasks, and a 10ml pipette was used to prepare the working standards. (See Table I for the determination of the working standards for the calibration of the flame photometer).

1ml of each water sample was diluted into a conical flask containing 100ml of distilled water to produce a \times 100 dilution. The flame photometer was set to sodium analysis and working standards were calibrated for the known concentrations. Diluted water samples were transferred to small beakers to be analysed by the flame photometer. Sodium concentrations that fell outside the calibration curve were diluted by a further 100ml to produce a \times 100,000 dilution. Sodium concentrations were then calculated accordingly using the linear equations of the corresponding calibration curve.

Data were analysed using Minitab version 15.1.0.0. stepwise regression and regression analysis were chosen to elucidate the relationship between the abundance of <u>C.volutator</u> and other tidal variables measured (salinity of the ebb and flow of tide, amount of suspended solids present during the tidal cycle, immersion and exposure times of <u>C.volutator</u>

during a complete tidal cycle and the flow rate of the ebbing tide). Regression analysis is widely used in ecology and still considered a valuable tool for recognising relationships within the data (Whittingham et al. 2006). Stepwise Regression was employed to determine which of the variables had the strongest relationship to the abundance of <u>C.volutator</u>. Spearman's rank correlations were then performed on the variables that had the greatest effect on the abundance of <u>C.volutator</u> as a measure of statistical dependence between the two variables (Yule & Kendall, 1950).

RESULTS

Speed of the ebbing tide was taken in addition to other measurements as an adaption of the experiment in light of the conditions on the day of the experiment. (For a summary of <u>C. volutator</u> abundance and tidal conditions in the Kentish Stour Estuary, please see Table I for details).

The immersion times and the salinity values of the flowing of the tide had the closest relationship to the abundance of <u>C.volutator</u> in comparison to all other variables. The relationship between the number of <u>C.volutator</u> and immersion time had a P value of 0.129 and salinity of the flowing tide had a P value of 0.146. As 0.29 and 0.146 > 0.05 the null hypothesis must be accepted as there is no statistically significant relationship between the abundance of <u>C.volutator</u>, immersion time and salinity of the flowing tide.

Spearman's rank correlations on the abundance of <u>C.volutator</u> and salinity of flowing tide showed weak positive correlation (P value 0.3363, df 1, Spearman's Rho 0.267) (Please see Fig 3. for details). The Spearman's rank correlation performed on the abundance of <u>C.volutator</u> and immersion time of <u>C.volutator</u> showed weak negative correlation (P value 0.9798, df 1, Spearman's Rho -0.007) (Please see Fig. 4 for details). Large error bars in both

graphs represent the large variability within the data. P values >0.05 indicate the absence of statistically significant correlation.

Further regression analysis on all tidal data showed that there was no statistically significant relationship between the abundance of <u>C.volutator</u> and all other tidal variables (F value 1.01, P value 0.435, df 6).

DISCUSSION

As no statistically significant relationship was identified between the abundance of <u>C.volutator</u>, and all tidal variables measured, it may be suggested that other confounding variables may have been causing an effect on Corophid distribution and abundance.

Contrary to McLusky's findings (1968), there appeared to be no significant relationship or correlation between the abundance of <u>C.volutator</u> and salinity of the tide. Presence of an old jetty east of the sampling site may have limited the saline penetration from the Atlantic Ocean by creating a physical barrier against the tide. This could have reduced the coalescing of salt water and freshwater, thus affecting the salinity measurements of the ebbing tide to a greater degree than that of the flowing tide.

Although no relationship was found between the speed of the ebbing tide and the abundance of <u>C.volutator</u>, burrows may have been shielded from the mechanical stimulation of waves by the jetty. This could have reduced the amount of wave exposure altering the influence of abiotic factors on the abundance and distribution of <u>C.volutator</u> (Drolet & Barbeau, 2008). An environmental impact assessment of the old jetty may therefore be beneficial to surveys carried out in this location in the future. Tidal flow rates vary with the direction of the prevailing winds and the angle of the slope of the river bank. As tidal speed is often dependant on the weather, measurements would have been more representative of the

site if taken over several days to produce an average tidal speed for the area. Tidal speed may have a direct correlation with Corophid distribution as organisms may be swept further down the slope of the mudflat by the strength of the tide. Pressure changes due to fluctuations in tidal flow can influence the zonal distribution of <u>C.volutator</u> (Morgan, 1965). Further investigation may require a measure of pressure to denote the relationship between the abundance of <u>C.volutator</u> and pressure changes of the water during the tidal cycle.

According to Kareiva & Odell (1987), the zonal distribution of <u>C.volutator</u> is reported to be a result of movement responses of <u>C.volutator</u> in reaction to habitat heterogeneity. Habitat heterogeneity is likely to be influenced by mudflat elevation as this can determine the effect of tidal exposure (Ysebaert et al. 2005). Mudflat elevation has been reported to influence the spatial patterns of <u>C.volutator</u> distribution and abundance, which could have had an effect on the abundance of Corophids sampled (Legendre & Fortin, 1989).

As samples were taken during a neap tide, tidal range was at a minimum. Close aggregations of Corophid burrows within the sampling band would have increased pore space of within the sediment thus increasing saturation time of the burrows upon immersion (Meadows & Tait, 1989). Mclusky (1968) reports that distribution and abundance of <u>C.volutator</u> is controlled by the nature of the substrate. A long term study investigating the relationship between the abundance of <u>C.volutator</u>, sediment composition and tidal variables of both neap and spring tides may offer further investigation into <u>C.volutator's</u> niche breadth limitations. It is suggested by McLusky (1970) that food may be necessary, at least in part, for osmotic regulation in <u>C. volutator</u>, as a direct supply of ions via the gut. Changes in sediment composition across the shore may therefore have been responsible for the zonal distribution of Corophid burrows. <u>C.volutator</u> is able to feed on organic matter on the sediment surface as a deposit feeder or on suspended particles by filter feeding (Ulrik-

Riisgård, 2007). The amount of organic matter present in the soil and suspended solids found within the tide may therefore limit the abundance and distribution of the species.

Predation disturbance can be an important determinant of invertebrate abundance in soft substratum communities such as the mudflats of Richborough Port (Kneib, 1984). As <u>C.volutator</u> is an important keystone species within intertidal mudflat ecosystems, predator – prey relationships may have had an effect on the abundance or distribution of the species (DeBacker et al 2009). Patterns of invertebrate distribution are often associated with the activities of aquatic predators (Kneib, 1984). Sandpipers feed almost exclusively on C.volutator where they are most abundant (Hicklin & Smith, 1984) increasing the potential for strong top down effects of trophic cascades (Wooton, 1994) As no predatory birds were witnessed around the sampling area, exclusion of predators should have led to an increase in Corophid abundance (Hanson & Kerekes, 2003). Veronica & Hughes (1994) suggested that abundance of C.volutator may be limited by the presence of primary produces in the form of benthic diatoms. Although, there were no sightings of shorebird predators, the wide distribution of the C.volutator burrows that formed the habitat band that was sampled from had the advantage of reducing competition and facilitating genetic diversity, leaving the species more adaptable to environmental changes (Hughes, 1988). The absence of predators suggests that there is little interference competition with <u>C.volutator</u> on the mudflats of Richborough Port however; exploitation competition may affect species abundances (Drolet et al. 2009).

Sampling was conducted for one day during the winter, which can be a time of high abiotic stress. Cold temperatures throughout the December season may have attributed to population declines in <u>C.volutator</u> prior to sampling. Cold weather may have influenced the distribution of Corophid burrows to drift towards the tide to reduce the effects of frost from the salinity of the water. <u>C.volutator</u> wintering generation have been reported to reproduce

once during the winter season, quickly dying off after reproduction with few animals surviving to produce a second brood (Flach, 1992). The sampled population of <u>C.volutator</u> were taken from the wintering generation where dramatic population declines may have occurred following reproduction, this may have dramatically influenced the abundance of Corophids sampled (Wilson & Parker, 1996). As data was only collected during one day it may not be representative of the dynamic behaviour of the ecological environment. Further replication across several days would have improved the reliability of the data, reducing the effect of post breeding mortality on the abundance of Corophids that were sampled.

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CAPTIONS

Fig. 1. Photograph of the sample site located at Richborough Port on the southern bank of the River Stour.

Fig. 2. Bird's eye view of <u>C.volutator</u> sample area situated on the intertidal mudflats on the bank of the River Stour at Richborough Port. Numbers within the grid represent the sample number. Letters indicate the replicate number for each sampling band.

Fig. 3. Spearman's rank correlations on the abundance of <u>C.volutator</u> and salinity of flowing tide.(P value 0.3363, Degrees of Freedom 1, Spearman's Rho 0.267).

Fig. 4. Spearman's rank correlation performed on the abundance of <u>C.volutator</u> and immersion time. (P value 0.9798, Degrees of Freedom 1, Spearman's Rho -0.007).

TABLE I

Amount of Distilled	Concentration/mg/l
Water/ml	
100	100
100	50
100	25
100	10
	Amount of Distilled Water/ml 100 100 100 100

Determination of the working standards for the calibration of the flame photometer.

 TABLE II

 Summary of Corophium volutator (Pallas, 1766) abundance and tidal conditions in the Kentish Stour Estuary

Sample Site	Abundance of Corophium volutator for every (L: 5.5cm × W: 4.2cm × H: 3.9cm) sampleMean ± S.D	Immersion Time (Minutes)	Exposure Time (Minutes)	Salinity of the ebbing tide Sodium Concentration (mg/l)	Salinity of the flowing tide Sodium Concentration (mg/l)	Suspended solids of the ebbing tide (g)	Suspended solids of the flowing tide (g)	Speed of the flowing tide (m/s ⁻¹)
1	9.67±10.60	807.00	633.00	0.28	0.32	0.054	0.023	0.43
2	12.33±4.16	811.00	629.00	0.28	0.32	0.05	0.237	4.74
3	12.33±208	813.00	627.00	0.27	0.32	0.005	0.011	2.20
4	9.67±3.06	816.00	624.00	0.28	0.38	0.039	0.054	1.38
5	11.00±3.00	824.00	616.00	0.28	0.38	0.044	0.042	0.95
6	24.00±9.54	828.00	612.00	0.29	0.38	0.066	0.086	1.30
7	10.67±3.51	831.00	609.00	0.24	0.38	0.013	0.131	10.08
8	8.33±2.52	836.00	604.00	0.29	0.37	0.054	0.131	2.43
9	15.67±4.16	840.00	600.00	0.29	0.37	0.018	0.114	6.33
10	17.33±7.37	850.00	590.00	0.29	0.37	0.025	0.062	2.48
11	14.00±5.00	883.00	557.00	0.24	0.37	0.016	0.107	6.69
12	14.33±2.08	926.00	514.00	0.25	0.37	0.048	0.116	2.42
13	16.67±4.93	959.00	481.00	0.25	0.37	0.053	0.125	2.36
14	6.67±3.51	961.00	479.00	0.24	0.36	0.022	0.108	4.91
15	4.33±4.04	1088.00	352.00	0.25	0.33	0.014	0.107	7.64

Fig. 1.

Study Area

Photograph of Sampling Site, located at 51°18'37"N 1°21'23"E.



Bird's eye view of <u>C.volutator</u> (Pallas, 1766) sample area situated on the intertidal mudflats on the bank of the River Stour at Richborough Port 51°18'37"N 1°21'23"E. Numbers within the grid represent the sample number. Letters indicate the replicate number for each sampling

Fig. 2.

The Rive	r Stour						
15a 15b 15c 🔘	Old Jetty						
14a 14b 14c 🌒							
13a 13b 13c		Stick					
0.3m 0.3m		12a	12ь	12c	•		
	Im	11a	11b	11c	•		
	0.2m	10a	10Ъ	10c	•		
	0.211	9a	9Ъ	9c	•		
Intertidal Mudflats		Sa	δр	8c			
		7 a	7b	7c	0		
		6a	6Ъ	6c	0		
		5a	5Ъ	5c	0		
		4a	4b	4c			
		3a	3Ъ	3c			
		2a	2Ъ	2c	0		
		1a	1b	1c	•		

band.

Fig. 3.

Spearman's rank correlation performed on the abundance of <u>C.volutator</u> (Pallas, 1766) and

salinity of flowing tide.

(P value 0.3363, Degrees of Freedom 1, Spearman's Rho 0.267).



Fig. 4.

Spearman's rank correlation performed on the abundance of <u>C.volutator</u> (Pallas, 1766) and

immersion time.

(P value 0.9798, Degrees of Freedom 1, Spearman's Rho -0.007).

