Helgol Mar Res (2015) 69:401–410
DOI 10.1007/s10152-015-0445-4

ORIGINAL ARTICLE

Microbial proliferation on gill structures of juvenile European lobster (*Homarus gammarus*) during a moult cycle

Karen L. Middlemiss[1] · Mauricio A. Urbina[1,2] · Rod W. Wilson[1]

Received: 20 June 2015 / Revised: 1 October 2015 / Accepted: 15 October 2015 / Published online: 31 October 2015

Abstract The morphology of gill-cleaning structures is not well described in European lobster (*Homarus gammarus*). Furthermore, the magnitude and time scale of microbial proliferation on gill structures is unknown to date. Scanning electron microscopy was used to investigate development of setae in zoea, megalopa and juvenile stages (I–V). Microbes were classified and quantified on gill structures throughout a moult cycle from megalopa (stage IV) to juvenile (stage V). Epipodial serrulate setae, consisting of a naked proximal setal shaft with the distal portion possessing scale-like outgrowths (setules), occur only after zoea stage III. After moulting to megalopa (stage IV), gill structures were completely clean and no microbes were visible on days 1 or 5 postmoult. Microbial proliferation was first evident on day 10 postmoult, with a significant 16-fold increase from day 10 to 15. Rod-shaped bacteria were initially predominant (by day 10); however, by day 15 the microbial community was dominated by cocci-shaped bacteria. This research provides new insights into the morphology of gill-grooming structures, the timing of their development, and the magnitude, timescale and characteristics of gill microbial proliferation during a moult cycle. To some degree, the exponential growth of epibionts on gills found during a moult cycle will likely impair respiratory (gas exchange) and ion regulatory function, yet further research is needed to evaluate the physiological effects of the exponential bacterial proliferation documented here.

Keywords Branchial grooming · Crustacean moulting · Epibiont control · Grooming morphology · Respiration · Bacteria

Communicated by H.-D. Franke.

✉ Karen L. Middlemiss
kombi@xtra.co.nz

✉ Mauricio A. Urbina
m.a.urbina-foneron@exeter.ac.uk; mauriciourbina@udec.cl

Rod W. Wilson
r.w.wilson@exeter.ac.uk

[1] Biosciences, College of Life and Environmental Sciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter EX4 4QD, UK

[2] Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Introduction

Gill structures in Crustacea contribute to several vital physiological processes such as respiration, osmoregulation, ion and pH regulation, as well as nitrogenous waste excretion (Henry et al. 2012). Decapod crustacean gills are complex structures which fall into three distinct morphological categories: phyllobranchiate, dendrobranchiate and trichobranchiate (Boxshall and Jaume 2009). In crabs (Decapoda) for instance, the gills are phyllobranchiate consisting of lamellar structures (Taylor and Greenaway 1979). Shrimp (e.g. penaeoid and sergestoid) possess dendrobranchiate gill structures (Boxshall and Jaume 2009). Crayfish and lobster (Decapoda) gills take on a trichobranchiate form with a central axis from which three columns of several filaments arise (rather than lamellae) creating a large surface area primarily to enable improved respiratory function (Dickson et al. 1991; Spicer and Eriksson 2003). As a result of bilateral symmetry in crustaceans, left and right branchial chambers are located on either side of the cephalothorax and protected by the

branchiostegite (an extension of the carapace). In *Homarus gammarus*, each gill chamber contains 20 trichobranchiate gills and 7 epipodites, both playing a role in respiration and osmoregulation (Haond et al. 1998; Lignot et al. 1999; Lignot and Charmantier 2001).

Preventing prolific fouling on these structures is vital in optimising physiological functions, which are also known to be reduced by aggregations of microbes contained within haemocytes at the gill, as part of an immune response (Schuwerack et al. 2001). Reduced physiological function therefore results from both internal and external bacterial development on the gills. Growth rates were significantly reduced, and resting oxygen consumption rates increased in the freshwater crab *Potamonautes warreni* after microbial infestations of the gills (Schuwerack et al. 2001). Bacterial development in the branchial chamber, as related to a moult cycle, is not well understood. A single study has previously investigated bacterial development in the branchial chamber of shrimp *Rimicaris exoculata* (Corbari et al. 2008). However, the magnitude and timescale of microbial proliferation on gill structures in *H. gammarus* has not been explored, nor characterised to date. Despite this gap in our knowledge, microbial control is known to be critical not only to the animal health, but also to increasing the chance of surviving environmental challenges such as changes in salinity (Urbina et al. 2010), oxygen levels (Paschke et al. 2010) and emersion (Urbina et al. 2013).

In an effort to control microbial pathogens present on specific external surfaces of the animal, decapod crustaceans utilise mechanical cleaning mechanisms, which include cleaning of olfactory, respiratory and sensory structures (Bauer 2013). Animals not possessing an exoskeleton (e.g. fish) have mechanisms that allow 'autocleaning' through adaptations such as the production of mucus by the integumental glands to prevent attachment by foreign substances (Bauer 1981, 2013). This adaptation does not apply to crustacean gills, and instead they possess other cleaning adaptations, i.e. periodic reversal of water flow through the gill chamber, and the passive or active use of setae and specialised appendages (e.g. chelipeds).

Gill-grooming mechanisms (passive or active) involve the use of complex setae possessing rasp-like structures to 'brush' foreign bodies off gill filaments. These mechanisms have been well described in larval and adult decapod crustacean species (*Disodactylus crinitichelis*, Pohle and Telford 1981), marine shrimps (Decapoda: Caridea, Bauer 1979, 1981), as well as clawed lobsters and crayfish (*Procambarus clarkii*, Bauer 1998; *Rimapenaeus similis*, Bauer 1999; *Homarus americanus*, Lavalli and Factor 1995). In *H. gammarus* gills, cleaning setae are attached to epipodites which are associated with the pereopods (legs) and maxillipeds (Haond et al. 1998). Movement of pereopods during normal locomotion/feeding causes repositioning of the epipodites, which in turn jostles the attached setae amongst the gill filaments. Setae are non-muscular structures and therefore rely on appendage locomotion, or water flow through the gill chamber to power their movement.

The effectiveness of gill-cleaning setae for microbial control is discussed by Bauer (1998) in research on crayfish *P. clarkii*. The author found that gill-cleaning setae are relatively ineffectual at removing epibionts during times of heavy infestation, however were effective at removing fouling by particulate matter (e.g. sediment). Conversely, active brushing of gill surfaces by the use of chelipeds inserted into the branchial chamber was shown to be highly effective at removal of both epibionts, and particulate matter in caridean shrimps (Bauer 1979). Most crustacean species identified, however, do not possess both passive and active cleaning mechanisms examined above (Bauer 1989), and *H. gammarus* has been described as possessing the less effective passive mechanism for microbial control. Microbial infestation of respiratory structures has the potential to negatively impact growth and survival; however, quantitative effects of microbial coverage of gill surfaces during a juvenile *H. gammarus* moult cycle have not previously been documented.

Neither the magnitude, nor the time scale of microbial proliferation, has been evaluated in crustacea early life stages during a complete moult cycle. Given the fragility of early life stages, a greater understanding of microbial development during frequent moults could lead to improved techniques for management of water quality in larviculture facilities, ultimately resulting in improved physiological processes during moulting. This would likely improve survival rates, welfare and overall production. It was hypothesised that despite the presence of a gill-cleaning mechanism (characterised in this study), microbial growth would increase during a moult cycle. The objectives of the present study were to (1) identify when setae first appear during the developmental stages, (2) describe the morphology of gill-cleaning setae and (3) provide the first report on magnitude, time scale and characteristics of microbial colonisation during a complete moult cycle in early *H. gammarus* development.

Materials and methods

Animals and experimental design

Animals were hatchery reared at ~20 °C in a salinity of 35 PSU (National Lobster Hatchery, Padstow, North Cornwall, UK). Zoea stage I–III animals were cultivated together in high density numbers and recognised as having moulted (within 24 h) through the use of expected time between stages, daily observation to identify phenotypic

changes of increased size or metamorphosis (stage IV), and confirmed by the presence of a newly moulted soft body, indicative of newly moulted animals. Zoea (stage III) animals were then transferred to individual cells for culturing, and therefore newly moulted megalopa (stage IV) and juvenile (stage V) animals (within 24 h) were easily identifiable from either shed exuvia in the culture cell (unless eaten), and/or new soft postmoult bodies. Morphology of both the gill structures, and epipodial setae in *H. gammarus*, was investigated using scanning electron microscopy (SEM) by sampling and fixing recently moulted larvae and juveniles ($n = 4$) at each stage as described above. Microbial development on immediate postmoult megalopa (stage IV) gills throughout the subsequent moult cycle to postmoult juvenile (stage V) was then assessed. Four animals were sampled at time points 1, 5, 10 and 15 days and then on day 1 (within 24 h postmoult) of moult to juvenile (stage V), approximately 17 days post-stage IV moult. Animals ($n = 20$ in total) were preserved in 2.5 % glutaraldehyde and kept at 4 °C until required for SEM (modified from Pohle and Telford 1981).

Scanning electron microscopy (SEM)

After removal of the carapace to expose gill structures, samples ($n = 4$) were fixed in 1 % osmium tetroxide for 24 h and then dehydrated in an ethanol series of 30, 50, 75, 90 and 100 % for 15 min at each concentration (a modified version of Boyde and Wood 1969). Samples were then critical point-dried (E3000, Polaron Equipment Limited, UK) using liquid carbon dioxide (CO_2), stub mounted and coated in gold palladium (20 nm thickness, sputter coater SC510, V.G. Microtech, UK) for analyses using a SEM (Jeol SEM 6390 at 5 kV acceleration voltage, magnification range 200–4000). Scanning electron microscopy was used to identify the structures associated with epipodial setae within the branchial chamber of stage I–V animals. For quantifying microbial development, images were also taken from each replicate and time point at 2000 × magnification from nine different locations on podobranch (outer layer) gills. These locations were the top, middle and bottom anterior bud on the first, third and fifth filament (anterior to posterior, respectively), in order to have better representation of microbial growth in the whole gills (Fig. 1a). Positions were selected on the outer layers of the gills because they were easiest to view, and because any removal of these structures during SEM preparation to gain access to inner gill structures may have resulted in damage, and/or removal of epibionts.

Left or right chambers were used depending on the quality (i.e. no damage during SEM sample preparation) and position and orientation of podobranch gills as identified using light microscopy prior to stub mounting. For image analysis, a 432 μm^2 macro-grid was created using ImageJ analysis software (ImageJ, v1.47). The grid was centrally overlaid over the image, on the base of each gill filament at the connection point with the central gill axis, and microbes counted within the grid (Fig. 1b). Microbes were only counted if their attachment point could be clearly seen within the grid. Microbes were counted and classified only as filamentous, cocci or rod-shaped, because accurate species identification could not be performed from images alone. Importantly, in addition to the specific images analysed, each gill filament was inspected in its entirety (for all animals) during SEM to ensure the reported results were representative of the whole gill filament condition.

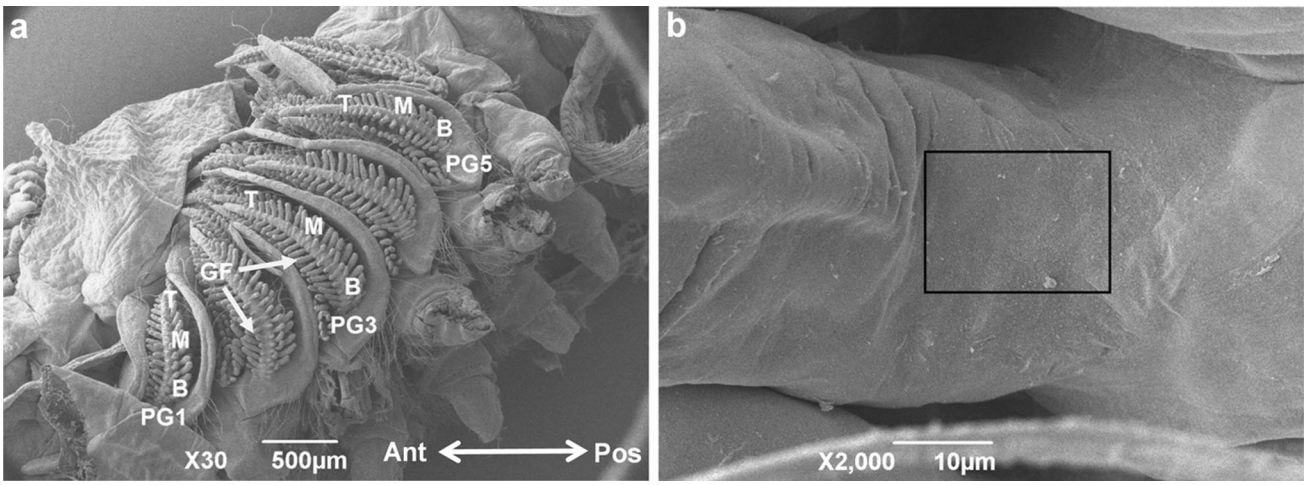

Fig. 1 *H. gammarus* juvenile stage V, **a** shows the nine sampling locations used for microbial counts on podobranch gills using SEM. Anterior/posterior position indicated with *arrows*. *PG* podobranch gill, *GF* gill filament, *B* bottom, *M* middle, *T* top. Images show each of the 9 GF sampling locations for microbial counting (**a**) and a representation of the positioning and relative size of a 432 μm^2 grid (marked by *black lines*) (**b**)

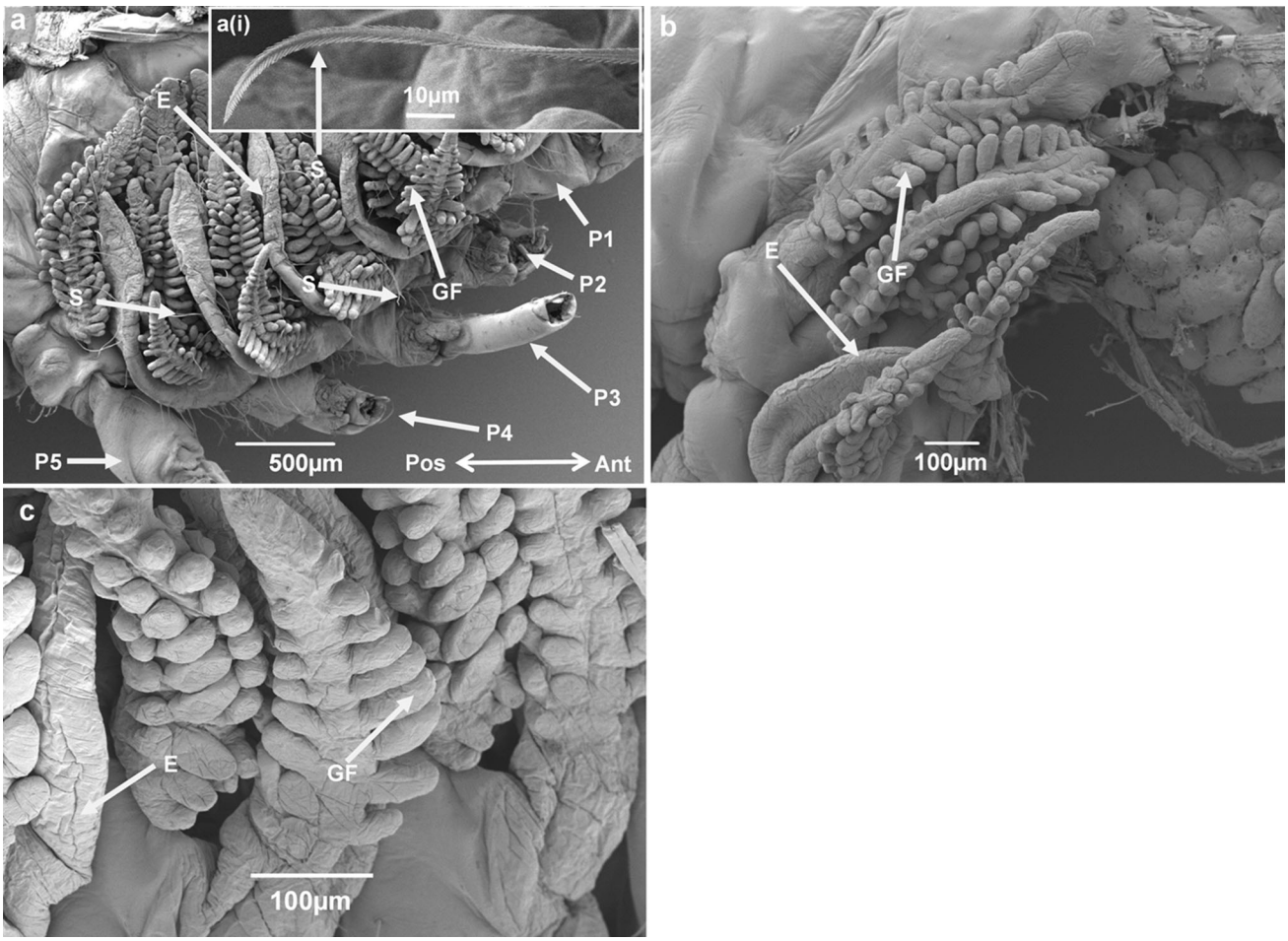

Fig. 2 Right branchial chamber of megalopa stage IV *H. gammarus* with branchiostegite (carapace) removed to reveal podobranch gills, gill filaments, epipodites and epipodial setae (**a**, **a**(**i**)). Anterior/posterior position indicated with *arrows*. P1–P5 pereopods have been removed to provide a clear view of the branchial chamber. The absence of setae on zoea stage I–II larvae is shown in (**b**) and (**c**), respectively. *PG* podobranch gill, *GF* gill filament, *E* gill epipodite, *S* serrulate setae

Data handling and statistical analysis

The terminology used throughout this paper to classify, describe and identify *H. gammarus* setal morphology is based on a review of the decapod setal classification system by Wortham et al. (2014), with particular attention to Jacques (1989), Watling (1989) and Garm (2004). All microbial counts from each animal (9 grids) were added together, and total microbial density (number/µm^2) was calculated as the total number of microbes divided by the total area sampled (i.e. 432 µm^2 per grid × 9 grids = 3888 µm^2 total area), providing one value per animal. Analysis was performed using SigmaPlot (v.11.0) and data presented as mean ± SE. In order to evaluate the progression of microbial proliferation during a moult cycle, microbial density (number/µm^2) was analysed using Kruskal–Wallis, as data failed assumptions of equal variances. Potential differences in the densities and time scale of growth of each bacterial type (number/µm^2) were analysed using ranked two-way ANOVA, and significant differences were subjected to a Tukey post hoc analysis. Significant differences were accepted at a $P \leq 0.05$.

Results

Developmental timing of setae and gill structures

Scanning electron microscopy of the right branchial chamber in *H. gammarus* revealed trichobranchiate gill structures separated by epipodites (flat blade-shaped structures) extending into the branchial chamber from the base of the pereopods (walking legs) (Fig. 2a). Gills are wider at the base and distally taper to a point, and each gill filament extends from a central axis. Setae are not present in the first two larval stages of *H. gammarus* (Fig. 2b, c). Setation was first observed at zoea stage III (Fig. 3c). In megalopa (stage IV) animals, serrulate setae were clearly

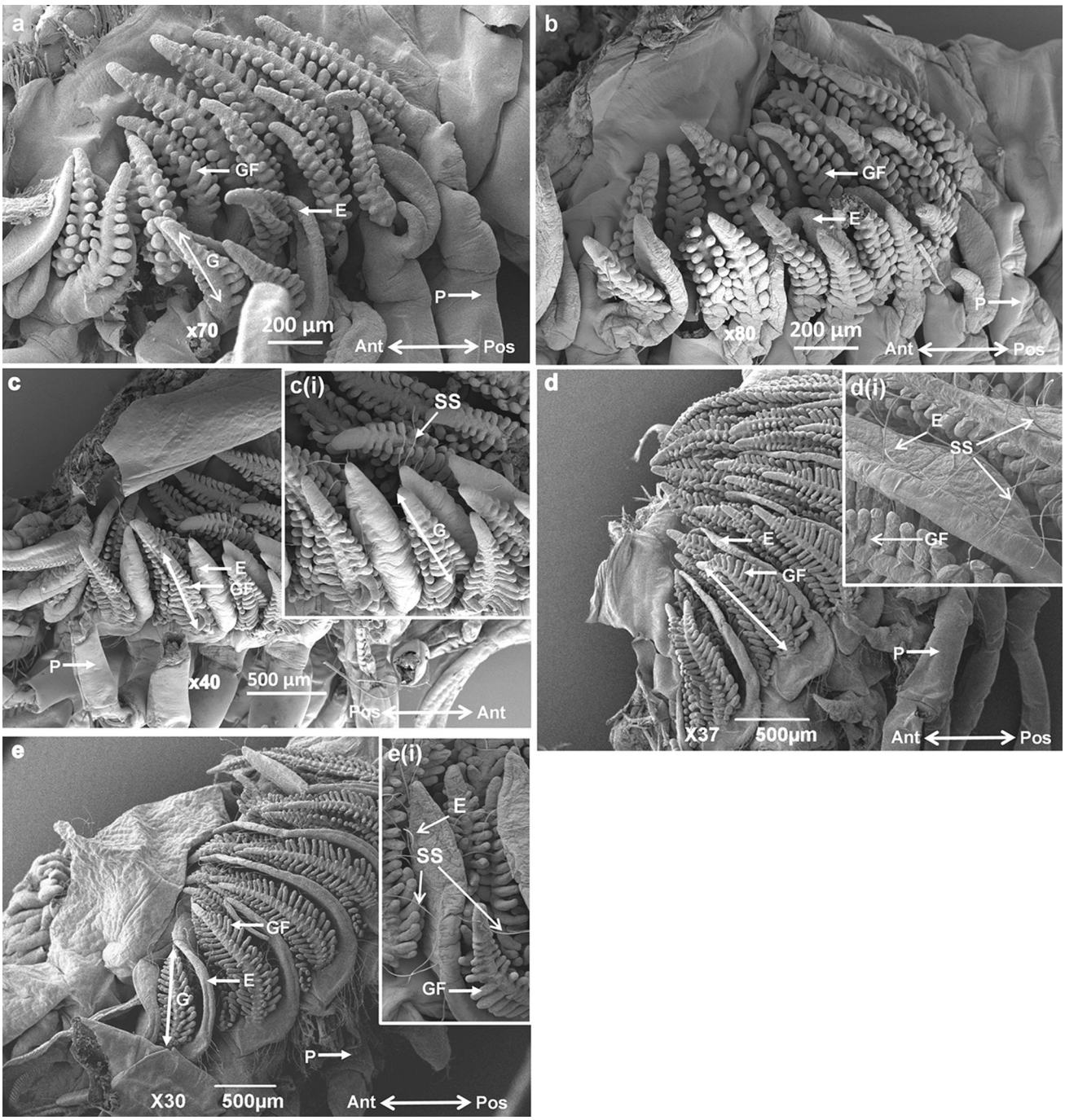

Fig. 3 Gill development and setal appearance in *H. gammarus* as shown by scanning electron micrographs in zoea larval stages I (**a**), II (**b**), III (**c**, **c**(**i**)), megalopa (IV) (**d**, **d**(**i**)) and first juvenile (V) (**e**, **e**(**i**)). Anterior/posterior position indicated with *arrows*. Setal appearance and development through zoea (stage III) (**c**), megalopa (stage IV) (**d**) and juvenile (stage V) (**e**). Serrulate setae structures have been enlarged in inset panels (**c**, **d**, **e**). *G* gill, *GF* gill filament, *E* gill epipodite, *P* pereopod, *SS* serrulate setae

visible and attached to epipodites positioned amongst the gills (Fig. 2a, a(i)). Morphological changes were evident as gill structures developed through the larval stages.

Both gill filaments and epipodites are present at the first stage of development (zoea I), but are small in size. Gill filaments present a rudimentary finger shape (Fig. 3a), and gills are separated from each other by an open space later occupied by fully grown epipodites (see below), and at zoea stage I do not occupy all available space in the branchial chamber. Gill filaments are short and roughly of the same length as the filament width. Epipodites are also short and are not located between the gills as in later larval

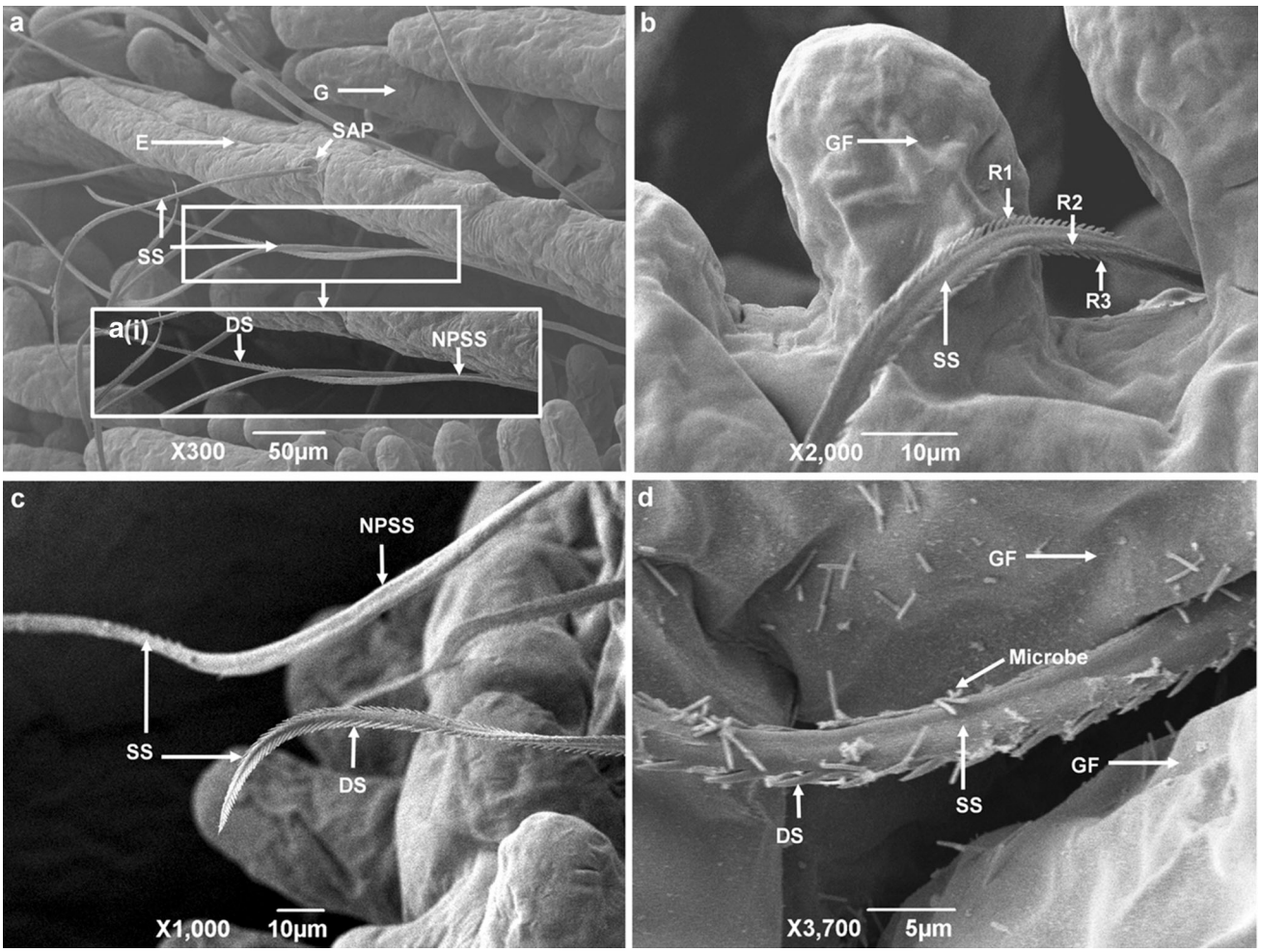

Fig. 4 Epipodial setae within the branchial chamber of *H. gammarus* showing microbial growth: serrulate setal structure showing setule outgrowths distal to the naked proximal shaft **a**, **a(i)**; three-sided setal structure (**b**); serrated distal and smooth proximal structure of setae (**c**); serrulate seta with setules showing epibionts attached (**d**). *SAP* setal attachment point, *GF* gill filament, *SS* serrulate setae, *E* gill epipodites, *DS* distal setules, *NPSS* naked proximal setal shaft, *R1*, *R2*, and *R3* rows of setules

stages (zoea III, megalopa (IV), and first juvenile (V); Fig. 3c–e). Gill structures continue to develop through the zoea II larval stage with filaments, and epipodites, becoming slightly larger and elongated as would be expected (Fig. 3b). No other structure or change was evident. This pattern of growth continues during the zoea III larval stage, again showing a small increase in size and length (Fig. 3c). Epipodites are now located between the gills, adopting the position of later stages. Also, at this stage epipodial setation is now evident. No setae were present in stages I–II (Fig. 3a, b), and development appears to be delayed until this third zoea stage (Fig. 3c, c(i)). Gills, associated gill filaments, and epipodites again become larger and elongated after moulting to megalopa (stage IV) with filaments appearing much longer than in previous stages, and setae are also longer and more abundant (Fig. 3d, d(i)). Megalopa (stage IV) and juvenile (stage V) gills and epipodites now appear fully formed (Fig. 3d, d(i), e, e(i)) with the epipodites elongated and fully extended into the top of the branchial chamber, as with the gills.

Setal morphology

Gill-cleaning setae are attached to the epipodites as previously shown in Fig. 3e(i)). These setal structures have a smooth shaft proximal to the epipodite attachment point, and distally three rows of setules circumscribing the shaft [tapering into a pointed tip) (setal attachment point (SAP); Fig. 4a, c]. The SAP is articulated, likely giving greater flexibility for the setae to move amongst the gills. Distally, the setae are three-sided (Fig. 4b) with setule outgrowths (Fig. 4c, d). The numerous setae are elongated (Fig. 3e) and positioned on the outer edge of the epipodites.

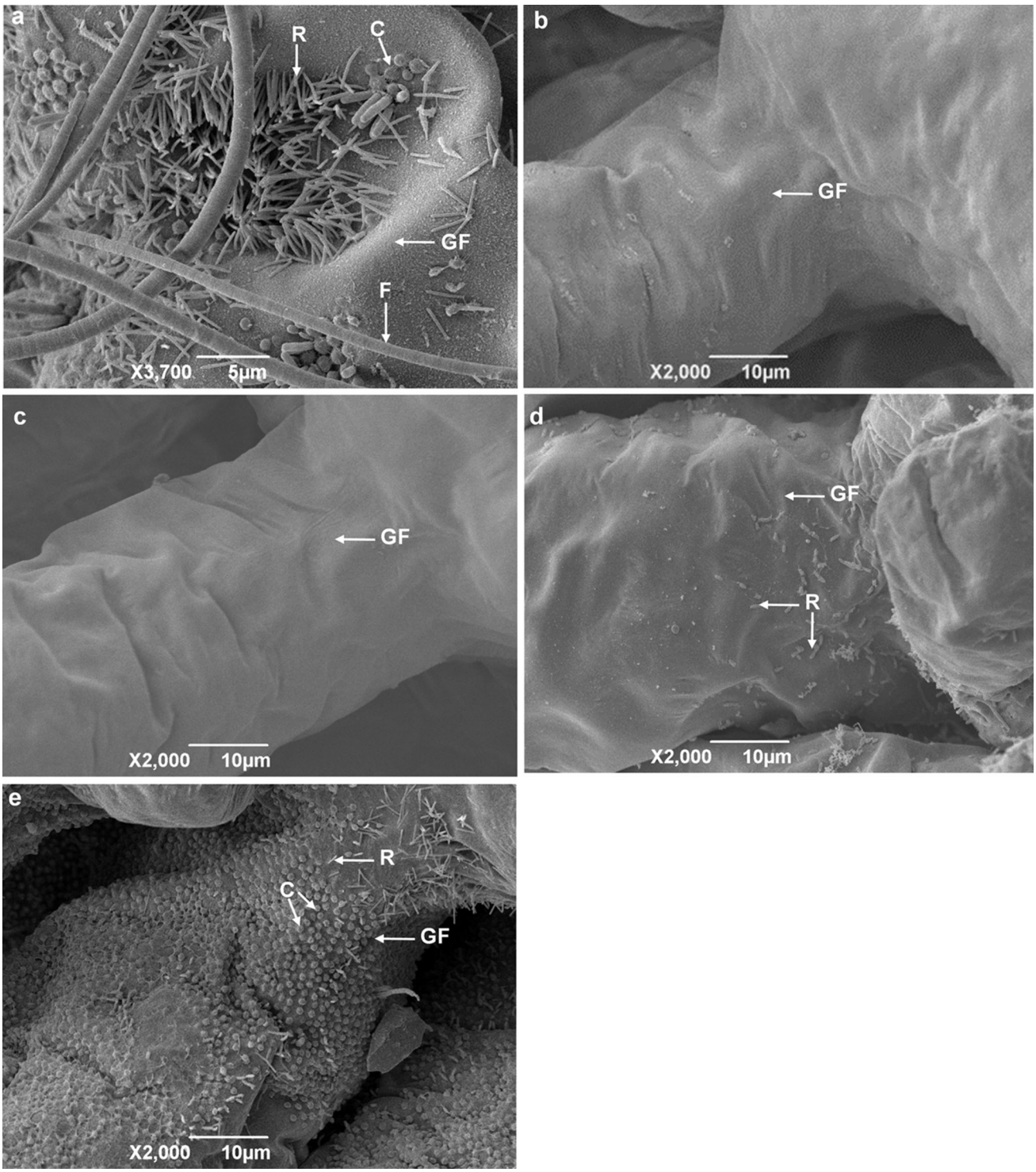

Fig. 5 Microbial growth on megalopa (stage IV) *H. gammarus* gill filaments classified by shape as either: rod; cocci; or filamentous (**a**). Images of microbial progression on megalopa (stage IV) gill filaments (1, 5, 10 and 15 days postmoult) (**b**–**e**). *R* rod, *C* cocci, *F* filamentous, *GF* gill filament

Microbial proliferation between moults

Microbes found on the gills of *H. gammarus* were categorised as either filamentous, cocci or rod-shaped (Fig. 5a), as described in the materials and methods section. Analysis of microbial development on *H. gammarus* gill surfaces between postmoult megalopa (stage IV) (days 1, 5, 10 and 15) and postmoult juvenile (stage V) (day 17) revealed a

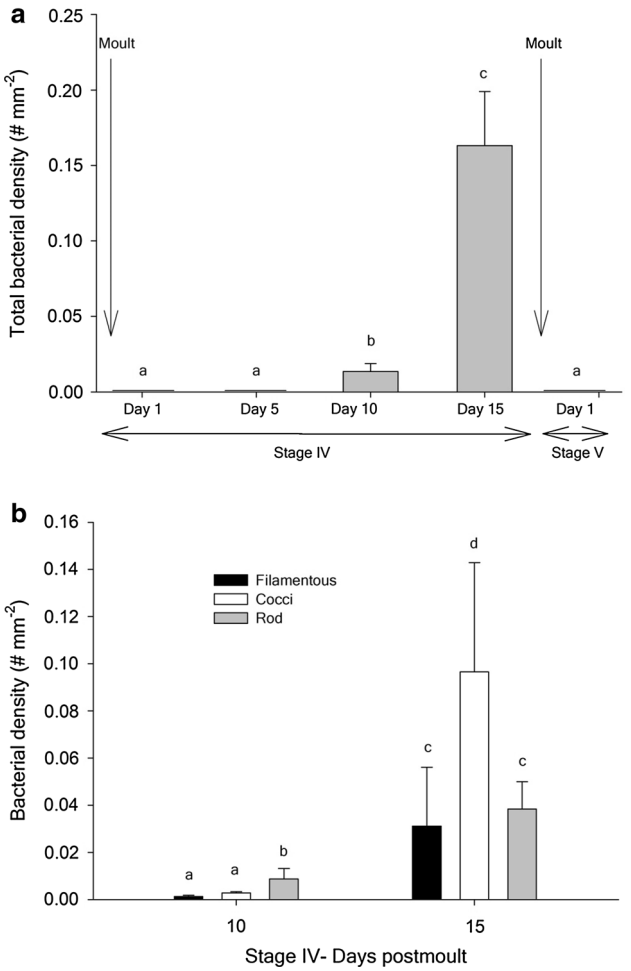

Fig. 6 Microbial counts during megalopa and juvenile (stages IV–V) moult cycles in *H. gammarus*: number of microbes per μm^2 ($n = 4$) on days 1, 5, 10 and 15 megalopa, and 1 day postmoult to juvenile (**a**); number of filamentous, cocci and rod-shaped microbes on days 10 and 15 postmoult ($n = 4$) (**b**). Data are presented as mean ± SE. Significant differences on and between the abundances of different microbes on days 10 and 15 postmoult are represented with different letters

clear cycle of changes. Microbes were not present on gill filament areas sampled on days 1 and 5 and were first visible and quantified at 10 days postmoult (megalopa stage IV) (Fig. 5b, c, d), with a further 16-fold increase in microbial density observed by day 15 (Tukey test, $P < 0.001$, Fig. 5e). Moulting to juvenile stage V removed all surface microbes, returning to a completely microbial-free cuticle as revealed on day 1 postmoult. At day 15 significantly higher numbers of all three types of microbes were present than at day 10 (Tukey test, $P < 0.001$, rod, cocci and filamentous, Fig. 6a). There were also significant differences in the abundance of the different types of bacteria (two-way ANOVA, $P < 0.05$). Rod-shaped bacteria were more abundant than filamentous bacteria 10 days postmoult (Tukey test, $P = 0.045$), while cocci-shaped bacteria were significantly more abundant than filamentous by 15 days postmoult (Tukey test, $P < 0.001$, Fig. 6b).

Discussion

Dramatic morphological changes were observed between *H. gammarus* larval stages in the development of gill-cleaning setae and associated gill structures, as well as the abundance and bacterial type present on gill surfaces at time intervals during a moult cycle.

Gill-grooming setae

Correct identification of setae involves the recognition of typical setal microstructures (i.e. denticles, setules and articulations). Images from the current study (zoea III larval stage onwards) clearly identified epipodial setae, classified as serrulate setae with setules (detailed definitions of setal structures found in Garm 2004). This is perhaps unsurprising given they are similar to that described for closely related *Homarus americanus* larvae (Factor 1978). Current results also showed an annulus present around the setal shaft, indicating the point at which setae were invaginated during ontogenesis (growth) (Mesce 1993; Watling 1989). Although annuli were seen in the images, this study did not focus on growth rates; therefore, setagenesis was not measured.

Epipodial setal development only occurs from zoea III larvae, just one stage prior to metamorphosis and subsequent migration to a benthic life cycle. This may suggest that control of microbial growth in earlier stages is not necessary with the use of setae. Passive gill-grooming setae provide control of sediment, but are comparatively less effective when compared to moulting, as shown through the use of ablation experiments by Bauer (1998). Although this study did not quantify the effectiveness of gill-cleaning setae, the magnitude and time scale of bacterial colonisation certainly validates previous ablation experiments suggesting their relative ineffectiveness at microbial control.

Absence of setae in the first two growth stages of *H. gammarus* may be a result of a high moult frequency during early developmental stages providing complete removal of microbes from gill structures after each moult. Similar to rearing conditions in the current study, *H. gammarus* growth during the first four life stages (zoea I–III, megalopa IV) occurred within an approximate 3-week period (Scolding et al. 2012). Animals used in the current study then moulted to juvenile stage V at around 31 days posthatch. Given the short growth period between moults (and considering microbial growth happens between 5 and

10 days postmoult in megalopa stage IV animals), it is suggested that setae are not required as a cleaning mechanism in these first two pelagic growth stages. Setae contribute to the removal of sedimentation and/or microbes; therefore, it is expected that the role of setae in gill grooming becomes particularly crucial during the settlement period from a pelagic to benthic environment. Our results support this theory with epipodial setae first present in zoea stage III animals and the last pelagic stage of *H. gammarus* prior to transition to the juvenile benthic stage.

The containment of gill lamellae or filaments within a confined branchial space increases the potential for trapping waterborne sediment or microorganisms (Bauer 1979). This is thought to be the driver for the development of grooming mechanisms in decapods, to control the negative impact of environmental (i.e. sediment) and biological (i.e. epibionts) factors on physiological functions (e.g. sensory, respiratory, pH and ion regulation). Passive gill-grooming mechanisms (the morphology of which is the primary focus in this current study on *H. gammarus*), related to epipodial setae attached to feeding and walking appendages, are activated by locomotion which in turn moves the setae amongst the gill structures. Bauer (2013) suggests, however, that not all 'passive' grooming is purely linked to normal locomotory/feeding movements and that the act of "limb rocking" (movement caused by the animal actively rocking backward and forwards on the pereopods) may be a specific attempt to engage the setae. Further studies would be required to determine if this mechanism were true of *H. gammarus*.

Microbial proliferation in the gills

Results showed that proliferation of microbes on the gills during a moult cycle is not immediate, taking longer than 5 days. Early stages of the moult cycle were populated predominantly by rod-shaped bacteria, whereas the end of the moult cycle was dominated by cocci-shaped bacteria. It is worthwhile noting that bacterial communities may be somewhat different in hatchery facilities compared to the natural environment, or those in the current study; however, the time scale and impact of microbial proliferation on *H. gammarus* gills should be comparable. It was also evident, although not quantified as part of this study, that passive epipodial setae were not able to prevent development of a rapidly increasing microbial community. This finding is supported by previous research into the effectiveness of gill-grooming setae in crayfish *Procambarus clarkia* and shrimp *Rimpenaeus similis* (Bauer 1998, 1999). Alternatively, and as expected, moulting is effective at complete removal of all microbes.

Comparatively large setule structures associated with gill-grooming setae (in relation to microbial size) are commonly thought to be unable to dislodge very small microbial organisms from gill surfaces. It is suggested that although setae of megalopa *H. gammarus* in the present study appeared ineffective at epibiont removal (given the exponential microbial growth rate on gill surfaces), they may play an important role in increasing the time it takes for proliferation to occur. If this is the case, then setae may also play an important role in increasing the time interval between moults and subsequently allow animals more time to gain energy reserves for the next moult by retarding the microbial colonisation of gills. The significance of needing to control gill microbes is related to the vital physiological processes involving these branchial structures, including oxygen consumption, pH balance and ion regulation. Gas exchange is impaired by the presence of epibionts on the gill surface via reduced surface area, and increased diffusion distance between the outer surface of the gill and the haemolymph, leading to decreased respiratory function (Schuwerack et al. 2001). Therefore, mechanisms for control of gill epibionts are critical to the health and survival of *H. gammarus*.

In summary, gill structures are rudimentary in early life stages of *H. gammarus* with gill-cleaning setae present only in zoea stage III onwards. These setae provide a mechanism to assist with maintenance of the various physiological functions of the gills (e.g. respiration), however appear ineffective at microbial control with the expected exponential development of microbes on gill structures throughout a moult cycle. However, setae could play an important role in reducing the time it takes for microbial proliferation of gill surfaces. Gill surfaces heavily colonised by microbes are less effective at carrying out functions such as respiration, which can result in impaired ability to fuel the increased demand needed to perform functions such as moulting. Furthermore, the type and abundance of the microbial community associated with gill structures changes during a moult cycle, posing several new questions about the consequences this may have on gill function. This could have important implications for improving water quality management in larviculture resulting in increased survival rates due to improved physiological function.

Acknowledgments The authors would like to acknowledge and thank the National Lobster Hatchery (Padstow, North Cornwall, U.K.) for provision of animals used in this research, and Mr Peter Splatt for bioimaging suite technical assistance (Exeter University, U.K.). We would also like to thank the anonymous reviewer/s for their valuable input into the draft manuscripts.

References

Bauer RT (1979) Antifouling adaptations of marine shrimp (Decapoda: Caridea): gill cleaning mechanisms and grooming of brooded embryos. Zool J Linnean Soc 65:281–303

Bauer RT (1981) Grooming behavior and morphology in the decapod Crustacea. J Crustacean Biol 1:153–173

Bauer RT (1989) Decapod crustacean grooming: functional morphology, adaptive value, and phylogenetic significance. In: Felgenhauer BE, Watling L, Thistle AB (eds) Functional morphology of feeding and grooming in Crustacea. Balkema, Rotterdam, pp 49–74

Bauer RT (1998) Gill-cleaning mechanisms of the crayfish *Procambarus clarkii* (Astacidea: Cambaridae): experimental testing of setobranch function. Invertebr Biol 117:129–143

Bauer RT (1999) Gill-cleaning mechanisms of a dendrobranchiate shrimp, *Rimapenaeus similis* (Decapoda, Penaeidae): description and experimental testing of function. J Morphol 24:125–139

Bauer RT (2013) Adaptive modification of appendages for grooming (cleaning; antifouling) and reproduction in the Crustacea. In: Watling L, Thiel M (eds) Functional morphology and diversity: 1 (the natural history of the crustacea). Oxford University Press, New York, pp 327–364

Boxshall GA, Jaume D (2009) Exopodites, epipodites and gills in crustaceans. Arthropod Syst Phylogeny 67:229–254

Boyde A, Wood C (1969) Preparation of animal tissues for surface-scanning electron microscopy. J Microsc 90:221–249

Corbari L, Zbinden M, Cambon-Bonavita M-A, Gaill F, Compere P (2008) Bacterial symbionts and mineral deposits in the branchial chamber of the hydrothermal vent shrimp *Rimicaris exoculata*: relationship to moult cycle. Aquat Biol 1:225–238

Dickson JS, Dillaman RM, Roer RD, Roye DB (1991) Distribution and characterization of ion transporting and respiratory filaments in the gills of *Procambarus clarkii*. Biol Bull 180:154–166

Factor JR (1978) Morphology of the mouthparts of larval lobsters, *Homarus americanus* (Decapoda: Nephropidae), with special emphasis on their setae. Biol Bull 154:383–408

Garm A (2004) Revising the definition of the crustacean seta and setal classification systems based on examinations of the mouthpart setae of seven species of decapods. Zool J Linnean Soc 142:233–252

Haond C, Flik G, Charmantier G (1998) Confocal laser scanning and electron microscopical studies on osmoregulatory epithelia in the branchial cavity of the lobster *Homarus gammarus*. J Exp Biol 201:1817–1833

Henry RP, Lucu Č, Onken, H, Weihrauch D (2012). Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals. Front Physiol 3:1–33

Jacques F (1989) The setal system of crustaceans: types of setae, groupings and functional morphology. In: Felgenhauer BE, Watling L, Thistle AB (eds) Functional morphology of feeding and grooming in Crustacea. Balkema, Rotterdam, pp 1–14

Lavalli KL, Factor JR (1995) The feeding appendages. In: Factor JR (ed) Biology of the Lobster *Homarus americanus*. Academic Press, San Diego, pp 349–393

Lignot JH, Charmantier G (2001) Immunolocalization of NA + , K + -ATPase in the branchial cavity during the early development of the European lobster *Homarus gammarus* (Crustacea, Decapoda). J Histochem Cytochem 49:1013–1023

Lignot JH, Charmantier-Daures M, Charmantier G (1999) Immunolocalization of Na + , K + -ATPase in the organs of the branchial cavity of the European lobster *Homarus gammarus* (Crustacea, Decapoda). Cell Tissue Res 296:417–426

Mesce KA (1993) Morphological and physiological identification of chelar sensory structures in the hermit crab *Pagurus hirsutiusculus* (Decapoda). J Crustacean Biol 13:95–110

Paschke K, Cumillaf JP, Loyola S, Gebauer P, Urbina M, Chimal ME, Pascual C, Rosas C (2010) Effect of dissolved oxygen level on respiratory metabolism, nutritional physiology, and immune condition of southern king crab *Lithodes santolla* (Molina, 1782) (Decapoda, Lithodidae). Mar Biol 157:7–18

Pohle G, Telford M (1981) Morphology and classification of decapod crustacean larval setae: a scanning electron microscope study of *Dissoodactylus crinitichelis* Moreira, 1901 (Brachyura: Pinnotheridae). B Mar Sci 31:736–752

Schuwerack PM, Lewis JW, Jones PW (2001) Pathological and physiological changes in the South African freshwater Crab *Potamonautes warreni* Calman induced by microbial gill infestations. J Morphol 77:269–279

Scolding JWS, Powell A, Boothroyd DP, Shields RJ (2012) The effect of ozonation on the survival, growth and microbiology of the European lobster (*Homarus gammarus*). Aquaculture 364–365:217–223

Spicer JI, Eriksson SP (2003) Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial postlarvae in the Norway lobster *Nephrops norvegicus* (L.)? J Exp Mar Biol Ecol 295:219–243

Taylor HH, Greenaway P (1979) The structure of the gills and lungs of the arid-zone crab, *Holthuisana* (Austrothelphusa) *transversa* (Brachyura: Sundathelphusidae) including observation on arterial vessels within the gills. J Zool 189:359–384

Urbina MA, Paschke K, Gebauer P, Chaparro OR (2010) Physiological energetics of the estuarine crab *Hemigrapsus crenulatus* (Crustacea: Decapoda: Varunidae): responses to different salinity levels. J Mar Biol Assoc UK 90:267–273

Urbina MA, Paschke K, Gebauer P, Cumillaf JP, Rosas C (2013) Physiological responses of the southern king crab, *Lithodes santolla* (Decapoda: Lithodidae), to aerial exposure. Comp Biochem Phys A 166:538–545

Watling L (1989) A classification system for crustacean setae based on the homology concept. In: Felgenhauer B, Watling L, Thistle A (eds) Functional morphology of grooming and feeding appendages, crustacean issues 6. Balkema, Rotterdam, pp 15–26

Wortham JL, VanMaurik LN, Price WW (2014) Setal morphology of the grooming appendages of *Macrobrachium rosenbergii* (Crustacea: Decapoda: Caridea: Palaemonidae) and review of decapod setal classification. J Morphol 275:634–649