Evolution des olfaktorischen Systems der Isopoda: Einblicke aus Neuroanatomie und Ethologie von *Saduria entomon* (Valvifera) Linnaeus 1758

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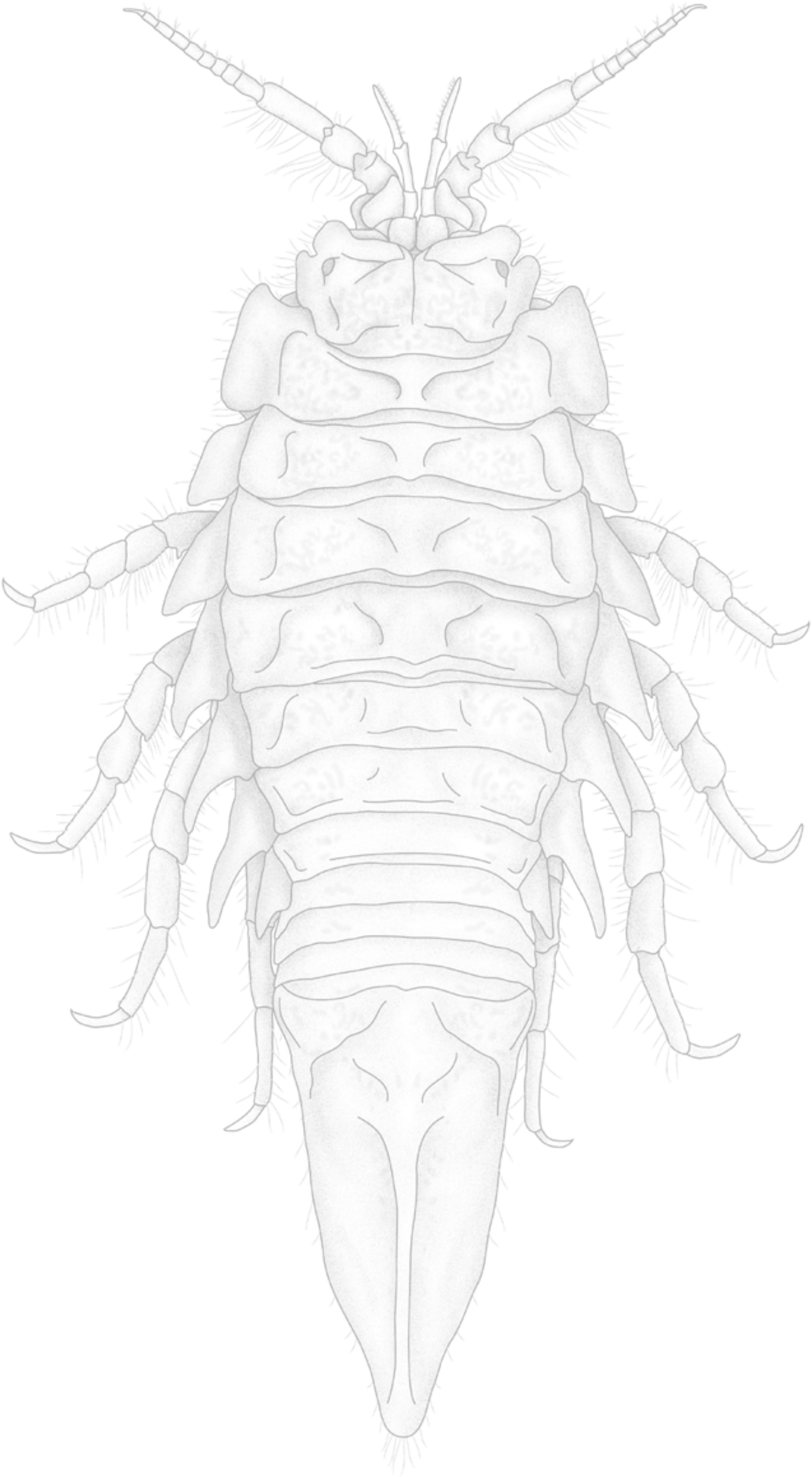

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I. Zusammenfassung

In einer Welt durchsetzt mit Gerüchen, haben marine Tiere hochentwickelte chemosensorische Systeme entwickelt um den vielfältigen Anforderungen des Lebens und Überlebens gerecht zu werden. Nahrungserwerb, Kommunikation, das Erkennen von Räubern oder potentieller Partner sind in diesem Kontext nur als Rahmen zu nennen. Durch eine Vielzahl an Sensillen, sowie durch spezifische, olfaktorisch geführte Verhaltensweisen, wie dem *antennal flicking* oder Stimulus-gerichteter Navigation, zeigen viele Vertreter der Malacostraca ein hohes Maß an Präzision und Genauigkeit in der Differenzierung und Lokalisierung von Düften. Die Mehrzahl der detaillierten morphologischen und ethologischen Studien konzentrierte sich bislang jedoch auf decapode Crustaceen. Das außer Acht lassen kleinerer Spezies abseits der klassischen Modellorganismen führte daher zu einer gewissen Einseitigkeit unseres Verständnisses der chemosensorischen Pfade und Nahrungssuchstrategien.

Während einige der terrestrischen Asseln (Oniscidea) schon gelegentlich als Vorlage für Studien dienten um die chemosensorischen Pfade in puncto Morphologie, Physiologie und Verhalten zu untersuchen, beruht unser Verständnis der chemischen Ökologie mariner Isopoden lediglich auf vereinzelten Beobachtungen und Annahmen. In der vorliegenden Arbeit sollen verschiedene Aspekte der Morphologie und Phänomenologie der Chemorezeption der baltischen Riesenassel *Saduria entomon* (Valvifera) LINNAEUS 1758 berücksichtigt werden. Abschließend soll anhand der vorgelegten Ergebnisse ein Rahmen entworfen werden, in welchem die Terrestrialisierung der Oniscidea neu betrachtet werden muss.

Gestützt durch 3D Rekonstruktionen, konventionelle Lichtmikroskopie sowie konfokale Laser-Scanning-Mikroskopie, wurden die generelle Anatomie des Gehirns, sowie das neuronale Substrat der chemosensorischen Pfade untersucht. Während es innerhalb der terrestrischen Isopoden zu einer drastischen Größenreduktion ihrer ersten Antenne und allen mit dieser assoziierten Gehirnareale kam, besitzt *S. entomon* ein olfaktorisches System, das in Bezug auf die antennale und neuronale Morphologie noch sehr grundmusternah aufgebaut ist. Im Vergleich mit den Decapoda zeigen sich jedoch deutliche Unterschiede in der strukturellen Diversität und dem Umfang von Nervengewebe das in der Verarbeitung chemischer Informationen beteiligt ist. Gleich ihren terrestrischen Verwandten zeigt *S. entomon* zudem einige Besonderheiten, die die sensorischen Pfade der zweiten Antenne betreffen. Die mikroglomeruläre Organisation des assoziierten Neuropils deutet auf eine zunehmende Bedeutung dieses Anhangs in der Wahrnehmung und Verarbeitung chemischer Informationen hin.

Verhaltensuntersuchungen lassen jedoch Zweifel an dem olfaktorischen Potential dieser Spezies aufkommen. Mittels eines Y-Labyrinthes und einer Reihe an Düften, dem das Tier in seiner natürlichen Umgebung begegnen mag, konnte gezeigt werden, dass *S. entomon* einen offenkundigen Mangel an Präzision aufweist, Stimuli zu differenzieren, sowie die Quelle eines Stimulus zu lokalisieren. In lediglich vier von 15 Experimenten ließ sich eine statistisch signifikante Verhaltensantwort beobachten. In diesen konnte darüber hinaus nur ein Stimulus als attraktiv identifiziert wurde.

Auf Basis von Freilandbeobachtungen, die das Tier mit einer gewissen Zufälligkeit umherwandernd darstellen, wurde ein Experiment entwickelt in welchem *S. entomon* in einem Mikrokosmos, und nur durch chemosensorische Sinne, einen Köder lokalisieren sollte. Obwohl es zwischen Kontrolle und Stimulusexperimenten deutliche Unterschiede in den aufgenommenen Bewegungsparametern gab, war kein von anderen Malacostraca oder Hexapoda bekanntes Suchmuster zu identifizieren. Eine statistische Auswertung der durch das Tier zurückgelegten Pfade ergab jedoch, dass die Tiere sich einer chemotaktischen Orientierung bedienten. Diese scheint zudem einer positiven rheotaktischen Bewegung überlagert. Um die Bedeutung der chemosensorischen Anhänge für eine erfolgreiche Nahrungssuche zu verdeutlichen, wurden chemische Ablationen der ersten und zweiten Antennen durchgeführt. Einige wenige Tiere waren zwar noch in der Lage den Köder zu lokalisieren, die Deaktivierung der Antennen führte aber zu einer beinahe vollständigen Unfähigkeit den Stimulus ausfindig zu machen. Eine Pfadanalyse konnte daher Chemotaxie als elementaren Orientierungsmechanismus ausschließen. Statt dieser wurde Chemokinesie mit einer ausgeprägten positiven rheotaktischen Komponente identifiziert. Darüber hinaus demonstriert dieses Experiment die Abhängigkeit *S. entomon*'s von der komplexen Interaktion der Distanz- und Kontaktchemorezeptoren für einen effizienten Suchlauf.

Bislang wurde davon ausgegangen, dass terrestrische Isopoden es nicht geschafft haben ihr olfaktorisches System derart anzupassen, dass es in Luft anstatt von Wasser operiert. Um der Notwendigkeit eines chemosensorischen Systems gerecht zu werden, entwickelte sich daher *de novo* ein System, in welchem die zweite Antenne sowie ihr neuronales Substrat entsprechend transformiert wurden. Das Vorhandensein eines gleichartig organisierten Systems in einem relativ nah verwandten marinen Vertreter deutet jedoch darauf hin, dass die Tendenz zu dieser Funktionstransformation der zweiten Antenne bereits im letzten gemeinsamen Vorfahren vorhanden war und somit der Kolonisation des Landes durch die Asseln vorausging. Die zweite Antenne als der maßgebliche chemosensorische Anhang der Oniscidea kann daher als Präadaptation verstanden werden, welche im Laufe ihrer Terrestrialisierung eine antennulare Olfaktion zweitrangig, wenn nicht sogar obsolet machte.

II. Summary

Living in a world full of chemicals, marine animals evolved elaborate chemosensory systems in order to find food and shelters, to communicate, as well as to assess the presence of predators or potential mates. Malacostracan crustaceans, decapods in particular, are considered as a paragon of a chemical detector. In being equipped with numerous chemosensory sensilla located on virtually every part of the body, and by applying specific behaviors like "sniffing" or stimulus directed navigation, many species display a high degree of precision and fidelity in detecting, differentiating, and localizing waterborne chemicals.

While most detailed morphological and ethological studies focused on decapod crustaceans, which moreover are mostly relatively large in regard to an odor plume, emitted from e.g. a prey item, our knowledge on chemosensory systems and food searching strategies shows a considerable bias, neglecting smaller species other than classical crustacean model organisms. Whereas member of the terrestrial Isopoda (Oniscidea) have repeatedly served as models to study the chemosensory pathways with respect to morphology, physiology, and behavior, our knowledge on the chemical ecology of marine isopods is close to zero.
The present work covers aspects of the morphology and phenomenology of chemoreception in the marine isopod *Saduria entomon* (Valvifera) LINNAEUS 1758, and offers a new frame in which the terrestrialisation by the Oniscidea has to be reconsidered.

Histological and immunhistochemical experiments were conducted using conventional light microscopy and confocal laser-scanning microscopy, corroborated by three-dimensional reconstruction in order to highlight the overall anatomy of the brain, as well as the neuronal substrate of the chemosensory pathways. Whereas terrestrial isopods have considerably reduced their first antenna, giving rise to a cascading effect on all associated brain areas, *S. entomon* features an olfactory system close to the malacostracan ground pattern in terms of antennal as well as neuronal morphology. In comparison with the Decapoda however, there are significant differences in the structural diversity and amount of neuronal tissue involved in processing chemosensory input. Like their terrestrial relatives, *S. entomon* shows some peculiar transformations regarding the sensory pathways of the second antenna, indicating an increased relevance in the perception and processing of chemosensory information by this appendage.

Behavioral experiments cast doubts on the olfactory potential of this species. Using a y-maze bioassay and a variety of odors the animal is likely to encounter in its natural habitat, *S. entomon* shows a considerable lack of precision in differentiating odors,

and in locating the source of an odor which otherwise is found to be quite attractive. In only four out of 15 experiments, a statistically significant behavioral response has been observed, and only a single Stimulus was found to be attractive.

Based on field observations depicting the animal wandering rather randomly through its habitat, a microcosm experiment was designed in which *S. entomon* had to locate a bait only by chemosensing. Although distinct differences in various movement parameters exist between control and stimulus treatment, *S. entomon* did not show any recognizable search patterns known from other Malacostraca or Hexapoda. However, a statistical evaluation of the animal's paths suggested chemotaxis as the elementary orientation mechanism. Moreover, this orientation might be superimposed on a positive rheotaxis.

In order to illustrate the importance of the major sensory appendages for successful foraging, chemical ablations of both, antenna one and antenna two were performed. Although some animals were still able to identify the source, the deactivation of the respective appendage resulted in an almost complete inability to locate the stimulus. Accordingly, path analysis excluded chemotaxis as elementary orientation mechanism for both cases but suggested chemokinesis with a distinct positive rheotactic component. Moreover, this experiment demonstrates that *S. entomon* is advised to a complex interaction of long distance- and contact-chemoreceptive appendages, i.e. antenna one and two, for efficient foraging.

It has hitherto been assumed that despite their success, terrestrial isopods have not been able to adapt an olfactory system to operate in air instead of water. Thus, in order to satisfy the need for a chemosensory system, they *de novo* developed a system in which the second antenna and its neuronal substrate have been transformed accordingly. The presence of a similarly organized system in a rather closely related marine representative now suggests that the tendency for a functional shift of the second antenna was already present in their last common ancestor and thus preceded the colonization of land by the Oniscidea. A distinctly chemosensory antenna two has therefore to be interpreted as a preadaptation that has rendered antennular olfaction secondary, if not even obsolete, during the terrestrialisation process.

1. Einführung

1.1 Zur Bedeutung der Chemorezeption

Die Fähigkeit Informationen aus der Umwelt auf- und wahrzunehmen ist eine fundamentale Basis für sämtliche ökologische Interaktionen von Organismen. Viele Tiere bedienen sich daher einer ganzen Reihe an unterschiedlichen sensorischen Pfaden, die verschiedene, zum Teil hoch spezifische Aspekte betreffen, und das Überleben und das Fortbestehen der Art sichern. Entgegen allen weiteren Reizmodalitäten und unabhängig vom umgebenden Medium sind sämtliche Interaktionen in irgendeiner Form mit *Düften* assoziiert. In einer Welt die regelrecht durchsetzt ist mit Chemikalien (im Kontext der *leaky bags* sensu Atema 1996), haben insbesondere aquatische Organismen wie Crustaceen hochentwickelte Systeme entwickelt, die es ermöglichen chemische Reize, olfaktorischer oder gustatorischer Natur, wahrzunehmen. Von der Verfügbarkeit von verschiedenen Ressourcen wie Nahrung oder eines Unterschlupfes, sowie von Substraten für die Besiedelung durch Larven, bis hin zur Klärung sozialer Hierarchien und dem Erfassen der Gegenwart von Prädatoren, Artgenossen oder potentieller Partner, gibt es keinen Bereich der durch diese Tiere nicht im Kontext der chemischen Ökologie erfasst wird (Schneider et al. 1999; Derby et al. 2001; Grasso und Basil 2002; Derby und Sorensen 2008; Breithaupt 2011; Thiel 2011; Weissburg 2011; Wyatt 2011).

Eine der wesentlichsten Aufgaben chemosensorischer Systeme ist daher die Wahrnehmung und Unterscheidung von Düften und Duftmixturen gegen einen äußerst variablen und aktiven Hintergrund. Während es an Land anhand der physikochemischen Eigenschaften vergleichsweise einfach ist eine Unterscheidung zwischen volatilen und anhaftenden Stimuli, sowie ihren entsprechenden sensorischen Organen zu treffen (d.h. Duft *versus* Geschmack, Olfaktion *versus* Gustation), verschwimmen diese Grenzen im aquatischen Milieu. Ein chemischer Stimulus soll (*sensu* Atema 1980) im Folgenden daher unabhängig von der Art der Perzeption, ganz generell als eine chemisch detektierbare Substanz verstanden werden. Soweit eine funktionelle Unterscheidung möglich ist, soll jedoch versucht werden zwischen Kontakt- und Distanzrezeption zu differenzieren.

Unser Verständnis der chemosensorischen Pfade der Malacostraca verdanken wir zu einem Großteil einer Handvoll Vertretern der Decapoda. Allen voran die Astacida (*Homarus*, Nephropidae, *Astacus*, Astacoidea, *Cherax*, Parastacoidea) und Achelata (*Palinurus*, Palinuridae) haben in dem noch recht überschaubaren Feld der chemischen Ökologie der Malacostraca regelrecht Modell-Status erreicht (Hay 2011). Zwar erlaubt uns dies einen beispiellosen Einblick in eine Tiergruppe, die als der Inbegriff eines chemischen Detektors verstanden werden kann, gleichermaßen wird dadurch aber auch die Einseitigkeit der Betrachtung anhand von klassischen Modellorganismen deutlich. Zahlreiche morphologische und ethologische Studien zeigen zudem, dass es innerhalb relativ nah verwandter Taxa nicht nur graduelle, sondern mitunter sehr drastische Unterschiede in der sensorischen Ausstattung, sowie den Fähigkeiten gibt, im Wasser gelöst Duftstoffe detektieren, differenzieren und lokalisieren zu können.

In der vorliegenden Arbeit soll daher auf eine weitere wichtige Gruppe der Malacostraca eingegangen werden - die Isopoda. Während es zu den Landasseln (Oniscidea) einige grundlegende Befunde gibt, die nahelegen, dass diese im Laufe ihrer Evolution anscheinend ihren Geruchssinn verloren oder zumindest zurückentwickelt haben, gab es bislang zu ihren marinen Vettern wenige verlässliche Informationen.

Im Mittelpunkt soll im Folgenden dabei die Baltische Riesenassel *Saduria entomon* (Valvifera, Chaetiliidae) LINNAEUS 1758 stehen, um drei zentrale Fragestellungen, die im Rahmen dieser Arbeit untersucht wurden, zu beantworten:

- Wie stellt sich die Architektur des Gehirns, sowie der peripheren und zentralen chemosensorischen Pfade eines marinen Isopoden dar? Welche Unterschiede können in den mit der Chemorezeption assoziierten Arealen zwischen marinen und terrestrischen Formen, sowie im Vergleich mit den Decapoda, identifiziert werden?
- Kann mittels eines Verhaltensexperimentes zum grundlegenden Unterscheidungsvermögen von Gerüchen untersucht und dargestellt werden, ob und wie Isopoden auf im Wasser gelöste Duftstoffe reagieren und durch diese angezogen und/oder abgestoßen werden? Welche Stoffe, Stoffkombinationen und Konzentrationen können als aktiv identifiziert werden? Inwiefern lässt sich das Verhaltensprofil mit bekannten Verhaltensantworten von Decapoden vergleichen?
- Welche Navigations-/Orientierungsstrategien und Mechanismen können identifiziert werden, die zur Lokalisation eines Stimulus genutzt werden, und wie fügen sich diese in das Gesamtbild zur chemischen Ökologie der Isopoda?

1.2 Die Isopoda

Mit rezent mehr als 10.000 beschriebenen Arten stellen die Isopoda eines der artenreichsten Taxa innerhalb der Crustaceen dar. Im Laufe ihrer mehr als 300 Millionen Jahre zurückreichenden Stammesgeschichte haben sie nahezu jedes Habitat des Planeten kolonisiert, angefangen von den flachen Schelfregionen der Meere, hinab in die abyssalen Tiefseeebenen, bis in die wasserlosen Wüsten des Landes (Brusca und Wilson 1991; Brandt 1992; Schmalfuss 2003; Schmidt 2008; Poore und Bruce 2012). Es verwundert daher wenig, dass diese Artengruppe eine große ökologische Vielfalt und eine ganze Fülle an Lebensformtypen hervorgebracht hat. Neben Aasfressern finden sich Herbi- und Detrivore, aber auch opportunistische Kannibalen, Parasiten jedweder Nuance, sowie Räuber. Gleichermaßen vielseitig stellt sich die Größe dieser Tiere dar, die nicht selten eponymischen Charakter hat. So finden sich im Interstitial der Süßgewässer und Meere die nur wenige hundert Mikrometer großen Microcerberidea. Demgegenüber steht die in den lichtlosen Tiefen der Ozeane beheimatete *Bathynomus giganteus,* die eine Gesamtlänge von beinahe 50 cm erreichen kann, eine Folge des abyssalen Gigantismus. Im Allgemeinen stellen sie sich als dorsoventral abgeflachte Tiere dar, die in der Dorsal- wie Frontalansicht einen ovalen Körperumriss aufweisen. Im Unterschied zu anderen Peracariden, insbesondere jedoch zu Amphipoden, sind sie daher oft relativ breiter und können sich dadurch flach an das Substrat anschmiegen. Ein charakteristisches Merkmal das diesen Tieren ihren Namen der *Gleichfüßer* gegeben hat, ist die Struktur der Peraeopoden. Sie sind sämtlich gleichartig und dienen, mit Ausnahme spezifischer Anpassungen an bestimmte Lebensweisen, als Laufbeine.

Wesentliche Untersuchungen zur Ökologie und Morphologie, und vereinzelt auch zum Verhalten von Isopoden, wurden maßgeblich an ihren terrestrischen Vertretern durchgeführt, den Oniscidea. Ein Umstand der maßgeblich darauf zurückzuführen sein dürfte, dass die Tiere in den meisten Fällen direkt vor der Tür aufgelesen werden können, marine Vertreter dagegen nur mit einem gewissen logistischen und technologischen Aufwand. Es zeigt sich daher aber gleichermaßen eine nicht zu unterschätzende Einseitigkeit in der Betrachtung dieses Taxons. Terrestrische Isopoden zeigen einige, zum Teil gravierende Abwandlungen und Modifikationen, die vielfach, wohl aber nicht exklusiv, mit der Besiedelung des Landes in Verbindung gebracht werden können.

1.3 Phylogenie der Isopoda

"*The structure of the Isopoda is so diversified, and the number of forms included in the Order is so large, that their classification is a matter of some difficulty*". Bereits vor mehr als 100 Jahren formulierte Calman (1909) diese Aussage zur Systematik der Isopoda die so treffend den Kern dieser Problematik trifft, und an welcher sich auch in den folgenden Jahren und Jahrzehnten wenig geändert hat.

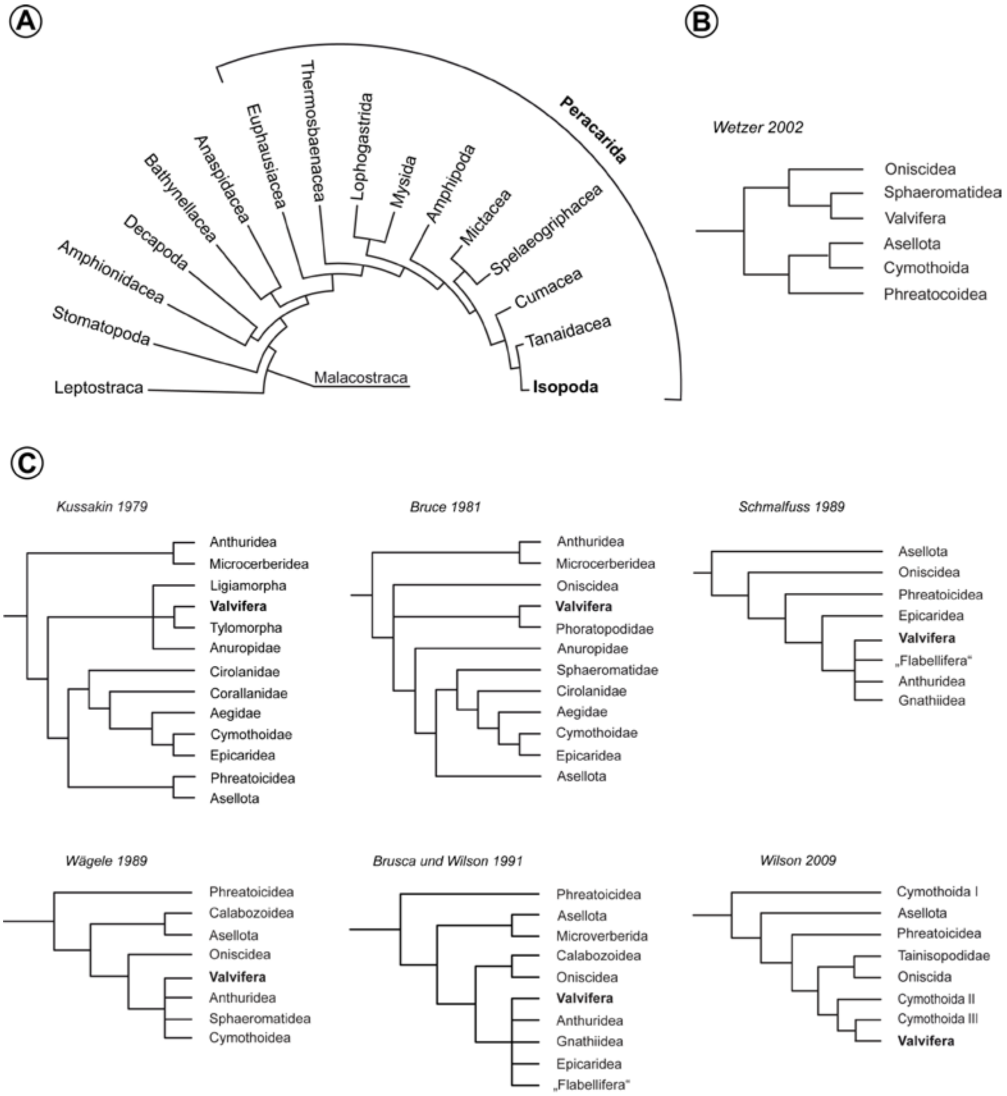

Abbildung 1.1 | Phylogenie der Malacostraca und Hypothesen zu den Verwandtschaftsverhältnissen innerhalb der Isopoda.

A Die Phylogenie der Malacostraca wurde nach Richter und Scholtz (2001) und Wirkner und Richter (2010) kompiliert. **B** Phylogenie der Isopoda nach Wetzer (2002), die soweit es phylogenetische Aspekte betrifft, den weiteren Überlegungen der vorliegenden Arbeit zugrunde liegt. **C** Diese Auflistung verfolgt nicht den Anspruch auf Vollständigkeit, sondern soll lediglich die Fülle an konkurrierenden Hypothesen und die mehrheitlich schlechte Auflösung der Stammbäume veranschaulichen.

So divers diese Artengruppe sich also darstellt, so vielseitig sind die Schlussfolgerungen und Annahmen zu den Verwandtschaftsbeziehungen (Vergleich Abb. 2.2). Denn, obgleich die Monophylie der Isopoden durch eine Reihe morphologischer Merkmale wohl begründet ist, sind sowohl die Stellung der Isopoda innerhalb der Peracarida und mögliche Schwestergruppenverhältnisse, als auch die internen Verwandtschaftsverhältnisse nach wie vor Gegenstand von Untersuchungen und zahllosen Diskussionen. Dies liegt nicht zuletzt in der Fülle der Synonyme und Mehrfachbeschreibungen sowie unterschiedlichen methodischen Ansätzen begründet, vor allem aber in der Komplexität, der ökologischen und morphologischen Vielfalt der Gruppe.

Die Monophylie der zugehörigen Taxa ist in der Mehrzahl nicht zufriedenstellend belegt, so finden sich häufig Neu- und Umgruppierungen. Beispielsweise die Flabellifera, die gelegentlich und wiederkehrend als Taxon vorgeschlagen, letztlich als paraphyletisch aufgelöst (Wägele 1989; Brusca und Wilson 1991), und durch die Taxa Cymothoida, Limnoriidea, Sphaeromatidea, Phoratopidea und Tainisopidea ersetzt wurden (Brandt und Poore 2003). Die parasitischen Formen innerhalb der Cymothoida und die Anpassungen an ihre Lebensweise, mit zum Teil enormen morphologischen Modifikationen, Verzwergungen und Reduktionen, sind ein weiterer Grund für die häufig schlechte Auflösung rein morphologisch gestützter Stammbaumrekonstruktionen. Eine kombinierte Analyse von Wilson (2009) kommt sogar zu dem Schluss, dass die Cymothoida polyphyletisch sind. Konsens besteht zumeist in der Stellung der ebenfalls morphologisch hochgradig spezialisierten Phreatoicidea. Diese werden als der basale Vertreter der Isopoda angesehen (Wägele 1989; Brusca und Wilson 1991). „Basal" ist in diesem Zusammenhang als rein mechanistischer Terminus zu verstehen der die frühe Abspaltung des Taxons in der Stammlinie der Isopoda beschreibt. Doch ist auch diese Interpretation nicht unangefochten. Verschiedene Morphologie-basierte aber auch molekularbiologische Untersuchungen legen nahe, dass ihre Stellung als abgeleitet zu verstehen ist, und das Taxon wesentlich später aus der Stammlinie hervortrat (Schmalfuss 1989; Wetzer 2002; Wilson 2009).

Uneinigkeit herrscht gleichermaßen bezüglich der Stellung der Oniscidea. Die Monophylie des Taxons ist durch ein mehrheitlich schlüssiges Autapomorphiegerüst gestützt (eine ausführliche Zusammenfassung findet sich in Schmidt 2008), die Frage nach ihren nächsten Verwandten bleibt aber weitestgehend unbeantwortet. Nach verschiedenen Untersuchungen kann jedoch eine stärkere Assoziation von Oniscidea und Valvifera angenommen werden. Zusammen mit den Sphaeromatidea, Anthuridea und Cymothoidea werden sie in dem Taxon der Scutocoxifera zusammengefasst (Wetzer 2002; Schmidt 2008), diese Ansicht teilen Michel-Salzat und Bouchon (2000) und Schmalfuss (1989) jedoch nicht. Im Folgenden sollen insbesondere die Valvifera (lat. *valvae* = Flügeltür, siehe unten) sowie die Oniscidea (terrestrische Isopoda) im Fokus stehen.

Soweit es phylogenetische Aspekte betrifft, wird sich die vorliegende Arbeit an der von Wetzer (2002) vorgeschlagenen Phylogenie orientieren (Abb. 1.1B). Im Grunde gibt es keine Position in der Isopodenstammlinie, an welche das 81 Gattungen umfassende Taxon der Valvifera noch nicht platziert wurde. Doch legt die Pleotelson- als auch die Magenmorphologie eine eher abgeleitete Stellung nahe (Wägele 1989; Brusca und Wilson 1991; Poore 2001). Offensichtlich charakteristische Merkmale sind demnach zum einen das posteriad ausgezogene Pleotelson (Vergleich Abb. 1.2), sowie die biramen Uropoden, die lateral am Pleotelson inserieren, sich namensgebend einer Flügeltür gleich unter diesen falten und die Pleopoden umschließen. Das zentrale Untersuchungsobjekt der vorliegenden Arbeit *Saduria entomon* ist innerhalb der Valvifera ein Mitglied der Chaetiliidae, DANA 1848. Diese sind morphologisch äußerst divers, die Gattung *Saduria* verfügt jedoch noch über die meisten plesiomorphen Merkmale der Chaetiliidae (Poore 2001).

1.4 Habitat und Habitus - *Saduria entomon entomon*

Die Makro- und Ichthyofauna der sublittoralen Weichböden der Ostsee weisen eine außergewöhnliche Artenarmut auf. Dies mag zum Einen auf die kurze postglaziale Zeit seit der letzten Vereisung zurückzuführen sein, maßgeblich dürfte sie aber in der geringen Salinität von 4 bis 7 PSU begründet liegen (Segerstråle 1959; Andreasson und Peterson 1982). Die Artenzusammensetzung von Brackwassergemeinschaften erreicht typischerweise ein Minimum bei Salinitäten von 5 bis 8 PSU. Kinne (1971) hat hierfür den Begriff des *Horohalinikum* etabliert. Ein weiterer Grund sind die zum Teil weitreichenden Sauerstoffminimumzonen, die sub- bis anoxische, und vielfach schwefelwasserstoff-haltige Bedingungen aufweisen. Es finden sich nur wenige, stark spezialisierte Arten, die diesen Lebensraum nutzen können. Die benthische Makroinvertebratenfauna der nördlichen Ostsee mit ihren angrenzenden Gebieten wird demnach durch ein überschaubares Artengefüge charakterisiert. Allen voran sind dies der Depositfresser *Monoporeia affinis,* LINDSTRÖM 1855 (Gammarida) und sein Räuber *Saduria entomon entomon* (Leonardsson 1991). Das Subspeziesepitheton zeigt an, dass es sich um die baltische Unterform handelt, der Einfachheit halber soll im Folgenden jedoch nur von *S. entomon* die Rede sein. Das primordiale Glazialrelikt *S. entomon* ist zirkumpolar verbreitet und besonders zahlreich in der nördlichen Ostsee (Bothnischer Meerbusen, Åland und dem Schärenmeer, sowie dem Golf von Finnland), dem Schwarzen Meer, sowie verschiedenen borealen Frischwasserseen Skandinaviens (bspw. Vättern und Mjörn; Charlesworth 1957) beheimatet. Ausgehend vom Arktischen Ozean besiedelten die stenothermen Kaltwassertiere seit dem Rückzug des Eisschildes zu Beginn des Holozäns unter anderem die skandinavischen Gefilde (Ekman 1940; Segerstråle 1956; Segerstråle 1957; Demel und Mulicki 1958).

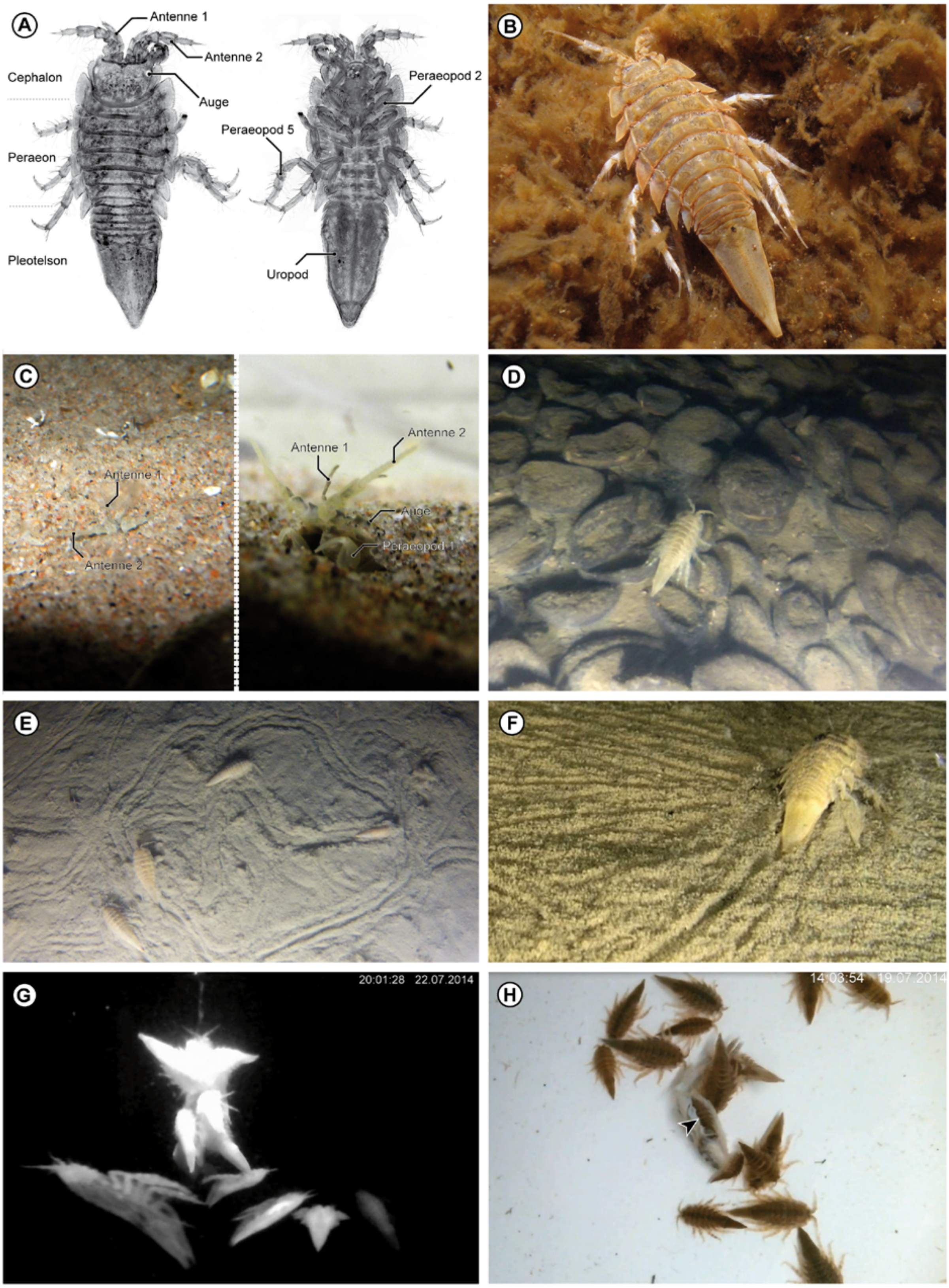

Abbildung 1.2 | Habitus und Habitat von *Saduria entomon*

A Habitus einer juvenilen *S. entomon*. **B** Habitus eines Adulten **C** *S. entomon* im Sediment vergraben und auf Beute lauernd. Nur die ersten und zweiten Antennen werden über den Boden gehalten. **D** *S. entomon* auf steinigem Meeresboden. Aufgrund des Mangels an für das Eingraben geeignetem Weichsubstrat ein recht untypisches Habitat. Dieser Fund verdeutlicht jedoch wie wenig über die Ökologie dieser Tiere bekannt ist. **E, F** Die Tiere hinterlassen charakteristische

◄ Spuren im Schlick. **G** Ein in das Hälterungsbecken gehängter toter Fisch (ca. 50 cm Höhe, Stint, *Osmerlus eperlanus*) wird einer Gruppe von *S. entomon* attackiert und gefressen. Es dauerte nur wenige Minuten bis die Tiere den Köder schwimmend erreichten. Auf diesen reagierten sie jedoch erst, als sie ihn mit einer ihrer Extremitäten berührten. **H** Eine Gruppe von *S. entomon* nagt an einem Stint (*O. eperlanus*). Häufig ist der gesamte Köder von einer Vielzahl an Tieren umgeben. Jede erreichbare Oberfläche wird genutzt um an Nahrung zu kommen, dabei kriechen die Tiere auch in geeignete Öffnungen hinein (Pfeil). *Fotos: A: Joachim Haug, UV-Autofluoreszenz; B: VELMU, Metsähallitus Parks & Wildlife Finland 2009, Essi Keskinen; D, E: VELMU, Metsähallitus Parks & Wildlife Finland 2012, ROV; F: SYKE 2009, Anna Downie.*

Die Spezies verfügt über einen ausgeprägten Größendimorphismus. Werden die Weibchen selten größer als 4 cm, erreichte das größte jemals verzeichnete Männchen eine Länge von 8,8 cm (Haahtela 1990). *Saduria entomon* stellt eine wichtige Nahrungsressource für Fische, wie verschiedene Groppenartige und Plattfische, vor allem aber junge Dorsche dar (Haahtela 1990; Sparrevik und Leonardsson 1998).

Nach Haahtela (1990) können die Tiere Abundanzen von über 120 Individuen pro Quadratmeter erreichen. Als vorwiegend omnivorer und opportunistischer Aasfresser, sind die Tiere daher aber auch ihrerseits einer der bedeutendsten wirbellosen Räuber der Nördlichen Ostsee. Zum Beutespektrum gehört dabei alles, dessen das Tier habhaft werden kann. Dies sind maßgeblich Larven der Chironomiden, Muscheln wie *Macoma sp.* und Crustaceen, wie dem zuvor genannten Amphipoden *Monoporeia*, dem Cladoceren *Daphnia* und anderen Isopoden wie *Asellus*. Diese werden zumeist im Sediment lauernd erbeutet, lediglich die ersten und zweiten Antennen verbleiben dabei über der Sedimentoberfläche (Green 1957; Leonardsson 1986; Sandberg und Bonsdorff 1990; Leonardsson 1991; Hill und Elmgren 1992; Bonsdorff et al. 1995; Ejdung und Elmgren 2001; siehe Abb. 1.2 C).

Nicht selten sind die Tiere in dieser Stellung entgegen ihren ansonsten scheinbar bedacht und träge anmutenden Bewegungen zu überfallartigen Vorstößen fähig um die Beute mit ihren ersten subchelaten Peraeopodenpaaren ergreifend. Dieser katapultartige Zugriff konnte mehrfach auch bei sich bewegenden Tieren an ausgelegten Ködern im Labor beobachtet werden. Die Tiere bewegen sich zumeist laufend fort, sind aber auch in der Lage über längere Distanzen auf dem Rücken liegend zu schwimmen (Abb. 1.2 G). Auf- und Vortrieb erreichen sie durch den Schlag mit ihren Pleopoden, die durch die aufgeschlagenen Uropoden freigelegt werden. Derart sind sie zu ungeahnt agilen Manövern fähig. Interessant ist hier die Stellung der Peraeopoden. Die letzten vier Beinpaare werden segelähnlich vom Körper abgespreizt, während die ersten drei dicht an den Körper herangezogen werden, jederzeit bereit zuzuschlagen. Fischereibiologische Untersuchungen gibt es diesbezüglich keine, doch wurde mir an der Zoologischen Station Tvärminne sowohl von Forschungstauchern als auch von Ansässigen mehrfach berichtet, dass *Saduria* in nicht unbeachtlicher

Zahl auch an in Netzen gefangenen Fischen fressend gefunden werden kann. Einen nicht zu unterschätzenden Einfluss auf die Artenzusammensetzung des Ökosystems, sowie auf die eigene Population übt *Saduria* durch einen ausgeprägten Kannibalismus aus (Leonardsson 1991; Sparrevik und Leonardsson 1998). Inwieweit sich dieser nur auf junge, schwache oder sich häutende Individuen beschränkt, ist unklar. Nikitina und Spasskii (1963) deuten jedoch an, dass vorrangig während des Winters jüngere Kohorten der Tiere in flachere Bereich migrieren, möglicherweise Ausdruck einer Räubervermeidungsstrategie in diesen ansonsten wohl nahrungsärmeren Zeiten.

Der Ursprung des Namens *Saduria,* ADAMS 1852 ist nicht eindeutig zuzuordnen. Wahrscheinlich von zweiteiliger Natur, ist die Herkunft von *Sad* oder eines Anagramms unbekannt, *uria* kann jedoch vom griechischen *oura* für Schwanz hergeleitet und als morphologisch deskriptiv verstanden werden. Das Artepitheton *entomon* ist ebenfalls zweiteilig und möglicherweise eine Referenz auf das Verhalten. Zum einen drängt sich die Ähnlichkeit zu *entoma* (*gr.* Insekt) auf, so werden die Tiere häufig als die Schaben der Ostsee bezeichnet. *Onos* steht wiederum für Esel oder Ramme. Zusammengesetzt ergibt sich eine recht treffende Beschreibung des Tieres, das sich einer Ramme gleich in den Weichboden bohrt und auf Beute lauert (Abb. 1.2 C), oder aber herumwandernd charakteristische Schleifspuren des langgezogenen Pleotelsons im Schlick hinterlässt, einem von einem Esel gezogenen Pflug nicht unähnlich (Abb. 1.2 E, F).

Typischerweise verbleibt *S. entomon* nahe der Sedimentoberfläche, aber auch Tiere in 10 cm Tiefe wurden beobachtet (Ejdung und Bonsdorff 1992). Marine Sedimente sind jedoch ab Tiefen von wenigen Millimetern nahezu sauerstofffrei und weisen darüber hinaus Sulfidkonzentrationen im Bereich von mehreren Millimol pro Liter auf (Fenchel und Riedl 1970; Nilsson und Rosenberg 1994). Ebenso können die Tiere bei der Nahrungsaufnahme dabei beobachtet werden, wie sie an einem Kadaver nagend an geeigneter Stelle hineinkriechen (Vergleich Abbildung 1.2 H). In Abhängigkeit der mikrobiologischen Aktivität sind auch hier alsbald anoxische Bedingungen gegeben. Neben einer effizienten Sulfiddetoxifikation ist eine der wesentlichen Anpassungen die *S. entomon* das Überleben in diesen Bereichen ermöglicht, und als einmalig innerhalb der Crustaceen gilt, ihr anaerober Glykolysestoffwechsel (Hagerman und Szaniawska 1990). Dieser kann auch in anhaltend sauerstoffarmen Bedingungen den Metabolismus aufrechterhalten (Hagerman und Oksama 1985; Hagerman und Szaniawska 1990). Obgleich *S. entomon* als nokturnal beschrieben wird (Westin und Aneer 1987), fanden Ejdung und Elmgren (2001), Johansson (1997) und ich während meiner Arbeit mit diesem Tier keinen offensichtlichen Unterschied in der Aktivität bei Dunkelheit und Licht, beziehungsweise Tag und Nacht.

1.5 Terrestrische Isopoda - die Oniscidea

Die Oniscidea gelten als die erfolgreichsten an das Landleben angepassten Crustaceen (Powers und Bliss 1983). Die knapp 3,600 Spezies bilden das einzige Taxon der Crustaceen, das sich beinahe ausschließlich aus strikt terrestrischen Arten zusammensetzt, und zählen neben den Insekten zu den häufigsten terrestrischen Makroinvertebraten. Wann genau und wie häufig Isopoden sich den Lebensraum Land eroberten ist aufgrund des fragmentarischen und häufig sehr schlecht erhaltenen Fossilberichtes nicht mit Endgültigkeit zu belegen. So wird vermutet, dass es, ähnlich der Situation innerhalb der Decapoda, mehrere voneinander unabhängige, häufig wohl weniger erfolgreiche, Kolonisationsversuche gegeben haben könnte (Vandel 1965). Die Wiege der Oniscidea kann auf den Zeitraum der späten Kreide bis frühen Paläogens (d.h. etwa ~100 - 60 Mio. Jahre) nur grob datiert werden (nach Broly et al. 2013). Zumindest vor etwa 40 Mio. Jahren waren alle modernen Familien vertreten und weit verbreitet. Ausgehend von der Morphologie und Ökologie heutiger marin-terrestrischer Zwischenformen, als Beispiel wird häufig die Gattung *Ligia* angeführt, wird angenommen, dass die ersten Oniscidea direkt dem Meer entstiegen und nicht den Umweg über Süßwasser genommen haben (Edney 1968; Tabacaru und Danielopol 1996; Hornung 2011). Derart erschlossen sie sich die trophische Nische abgestorbener, küstennaher Vegetation und stellen seither einen Großteil der terrestrischen Mesofauna und primären Destruenten (Shachak et al. 1976; Davis 1984; Gongalsky et al. 2005).

Diese Hypothese ist im Einklang mit der gängigen Theorie der Adaptation der Amphipoden (Hurley 1968). Zwar sind auch die Oniscidea aufgrund des Fehlens einer wasserabweisenden Cuticula nach wie vor an feuchte Mikrohabitate (wie Bauten, Spalten in Bäumen oder Felsen), oder entsprechende wasserkonservierende Lebensweisen (bspw. Nachtaktivität) gebunden (Warburg 1968; Edney 1968), doch haben sie im Laufe ihrer Evolution einige der anspruchsvollsten physiologischen Hürden des Lebens an Land gemeistert und sich maßgeblich von ihrem marinen Erbe gelöst. Sämtliche biologischen Aktivitäten, angefangen von Respiration über Thermoregulation, Osmoregulation und Exkretion, bis hin zur Reproduktion, erfolgen an Land. Ebenso ist im Gegensatz zu allen anderen terrestrialisierten Crustaceen während keiner der Entwicklungsstadien, vom Ei bis zum Juvenil, stehendes Wasser erforderlich. Besonders eindrücklich wird der Erfolg dieses Taxons aber dadurch dokumentiert mit Ausnahme der Polarregionen, jeden Winkel des Planeten, inklusive der Wüstenregionen, kolonisiert zu haben (Brusca und Wilson 1991; Schmalfuss 2003; Broly et al. 2013). Dieser Erfolg hatte jedoch zu einen Preis - demn funktionellen Verlust ihrer ersten Antenne und wohlmöglich sämtliche mit dieser assoziierten sensorischen Funktionen.

2. Morphologie

2.1 Die morphologische Basis der Chemorezeption

2.1.1 Das Gehirn der Malacostraca im Überblick

Der Aufbau des Gehirns der Malacostraca war Gegenstand überaus zahlreicher und detaillierter Untersuchungen (Strausfeld 2012; Loesel et al. 2013; Sandeman et al. 2014). Zur generellen Morphologie soll zunächst anhand des Grundmusters der Malacostraca nach Kenning et al. (2013) ein allgemeiner Überblick gegeben werden (Abb. 2.1). Die im Folgenden verwendete Terminologie orientiert sich an Sandeman et al. (1992), Harzsch (2002), Richter et al. (2010), sowie Loesel et al. (2013). Aufgrund der bilateralen Natur des Malacostracengehirnes beziehen sich die Beschreibungen soweit nicht anders vermerkt auf nur eine Hemisphäre. Auf die Organisation des ventralen Nervenstranges soll an dieser Stelle nicht weiter eingegangen werden.

Das Syncerebrum der Malacostraca entstand aus der Fusion von drei Neuromeren. Diese sind entsprechend der Neurachse von anterior nach posterior das Proto-, Deuto- und Tritocerebrum, und können morphologisch entsprechend ihrer innervierenden Anhänge, als auch auf Basis der Expression der Segmentpolaritätsgens *engrailed*, voneinander abgegrenzt werden. Ein wiederkehrendes und stark kritisiertes Konzept, nach dem das Protocerebrum aus einer Fusion des Archicerebrums mit dem Prosocerebrum hervorging, sei an dieser Stelle lediglich erwähnt (Ihle und Ihle-Landenberg 1939; Meyer 1986; Damen et al. 1998).

Das augentragende Protocerebrum lässt sich ferner in einen paarigen lateralen und unpaaren medianen Teil untergliedern. Ersterer ist bei vielen Crustaceen in den Augenstielen lokalisiert, bei Arten mit aufsitzenden Augen wie Isopoden ist er in die Kopfkapsel eingegliedert. Der visuelle Input der Komplexaugen, so vorhanden, wird jedoch unabhängig von der Lage in einer Serie von kolumnären, retinotopen Neuropilen prozessiert. Dies sind die vielfach auf komplexe Weise mittels zweier Chiasma miteinander verschaltete Lamina, Medulla, Lobula und Lobulaplatte. Ebenfalls Bestandteil des lateralen Protocerebrums sind die Neuropile des Hemiellipsoidkörpers und der Medulla Terminalis. Während der Hemiellipsoidkörper in Anlehnung an seine namensgebende Form mehr oder weniger klar, vor allem aber durch seine Innervationen und die ihm zugeschriebene Funktion definiert ist (siehe unten), umfasst die Medulla Terminalis recht unspezifisch „den Rest" des lateralen Protocerebrums. Im medianen Protocerebrum befindet sich zwischen den diffus organisierten und funktional wenig entschlüsselten anterioren und posterioren medianen protocerebralen Neuropilen, der Zentralkomplex. Verschiedene Funktionen werden diesem Komplex, bestehend aus Protocerebralbrücke, Zentralkörper und lateralen akzessorischen Loben, zugeschrieben.

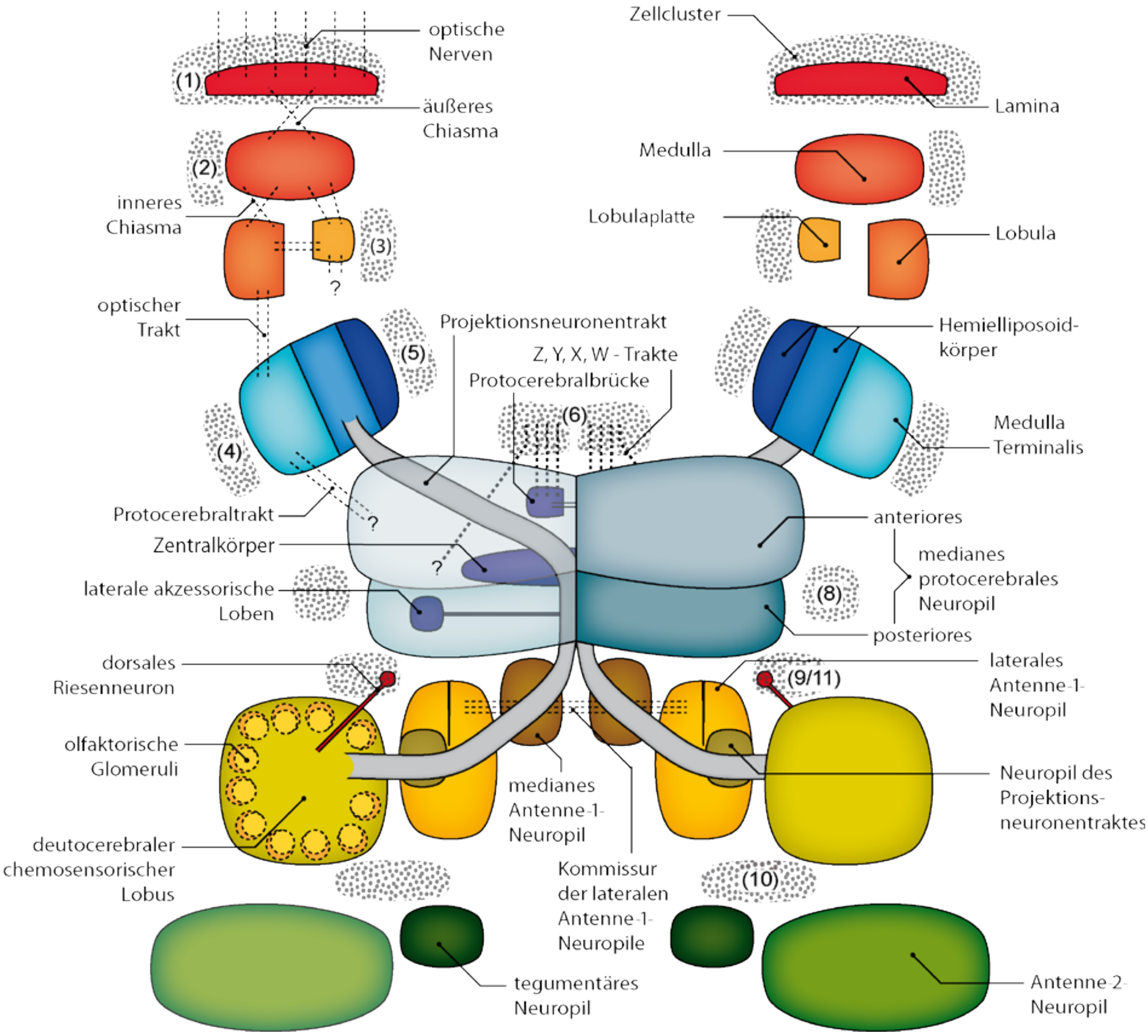

Abbildung 2.1 | Das Grundmuster der syncerebralen Organisation der Malacostraca. Von anterior nach posterior lässt sich das Gehirn der Malacostraca in die Neuromere des Protocerebrums (Rot und Blautöne), des Deutocerebrums (Gelb und Brauntöne) und des Tritocerebrums (grün) gliedern. Diese sind embryologisch über Segmentpolaritätsgene, oder funktionell durch ihre innervierenden Anhänge voneinander abgrenzbar. Das Protocerebrum ist, so vorhanden, mit den Augen assoziiert, das Deutocerebrum mit der ersten, und das Tritocerebrum mit der zweiten Antenne. Verändert nach Kenning et al. (2013).

Neben motorischer Kontrolle und parakrinen Funktionen, legen Verbindungen zum visuellen System eine integrative Verarbeitung entsprechender Stimuli nahe (Utting et al. 2000; Homberg 2008; Strausfeld 2012). In jedem Falle kann durch die sprich- und wortwörtliche Lage eine zentrale Rolle in Koordination und Integration von sensorischem Input und Vermittlung von entsprechendem Output angenommen werden. Das Deutocerebrum ist mit der ersten Antenne (Antennula) assoziiert und besteht aus dem deutocerebralen chemosensorischen (olfaktorischen) Lobus namensgebender Funktion, dem lateralen Antenne-1-Neuropil, dem medianen Antenne-1-Neuropil, und dem Neuropil des Projektionsneuronentraktes. Dieser Trakt umfasst eine Vielzahl von Projektionsneuronen, die ausgehend von einem prominenten Zellcluster die olfaktorischen Glomeruli mit den Neuropilen des lateralen Protocerbrums verbinden (Abb. 2.1 - 2.3). Einige decapode Krebse verfügen über ein weiteres Neuropil, den akzessorischen Lobus. Diese Neuerung innerhalb der Reptantia erfährt in den Coenobitidae und Brachyura wieder eine Reduktion, während er in den Astacidea einen Großteil des Deutocerebrum ausmachen kann. Er erhält chemo- und mechanosensorischen Input höherer Ordnung und ist, insbesondere beim Flusskrebs, ein wichtiges integratives Areal (Sandeman et al. 1995; Sandeman et al. 2014). Das mit der zweiten Antenne (Antenna) assoziierte Neuromer, das Tritocerebrum, besteht aus dem chemo- und mechanosensorischen Antenne-2-Neuropil und dem tegumentären Neuropil. Letzteres erhält mechanosensorische Afferenzen des Carapax.

2.1.2 Die peripheren chemosensorischen Pfade

Die Rezeptorneurone der Malacostraca sind bipolare Neurone, deren apikaler Dendrit mit einer externen cuticulären sensorischen Struktur - dem Sensillum - in Verbindung steht, während ihr basales Axon direkt in das zentrale Nervensystem projiziert (Hallberg und Skog 2011; Schmidt und Mellon 2011; Mellon et al. 2014). Ausgehend von ihrer Modalität können sie in Mechano-, Chemo- und olfaktorische Rezeptorneurone differenziert werden. Mechano- sowie Chemorezeptorneurone sind mit Abstand am zahlreichsten und innervieren bimodale chemo- und mechanosensorische Sensillen die nahezu über die gesamte externe als auch interne Körperoberfläche (bspw. Kiemenhöhlen) verteilt zu finden sind. Maßgeblich aber auf der ersten und zweiten Antenne, den Mundwerkzeugen, sowie den Dactyli der Laufbeine (Heinen 1980; Derby 1982; Derby und Atema 1982; Guse 1983; Pynnönen 1985; Tsuneo 1989; Derby und Steullet 2001; Garm et al. 2003; Garm et al. 2005; Hallberg und Skog 2011).

Die olfaktorischen Rezeptorneurone innervieren dagegen rein unimodale olfaktorische Sensillen, die ausschließlich auf dem lateralen Flagellum der ersten Antenne lokalisiert sind - die Aesthetasken. Diese werden ob ihrer Ausstattung mit ionotropen Rezeptorproteinen (Groh-Lunow et al. 2015) als das primäre Riechorgan der Malacostracen angesehen. Die zweite Antenne ist ebenfalls in unterschiedlicher Ausprägung chemosensorisch; diese wird jedoch als der maßgeblich mechanosensorische Anhang verstanden. Wie im Folgenden dargelegt werden soll, spielen die Isopoda in diesem Falle jedoch wieder eine gesonderte Rolle.

2.1.3 Die zentralen chemosensorischen Pfade

Über den Nerv der ersten Antenne projizieren die primären Afferenzen in das Deutocerebrum. Die Aesthetasken entsenden in die deutocerebralen chemosensorischen Loben, während die chemo- und mechanosensorischen Afferenzen im lateralen und medianen Antenne-1-Neuropil münden (siehe Abb. 2.1 - 2.3). Diese Aufteilung ist auf eine Zweiteilung der Pfade in der Verarbeitung chemischer Stimuli zurückzuführen (Schmidt und Mellon 2011).

Der erste Pfad, die Olfaktion, ist bislang am eingehendsten untersucht und beschrieben worden, und beschreibt die Aesthetasken-vermittelte Chemorezeption. Sie wird als eine Qualitätsbeurteilung verstanden, die ohne spezifischen Bezug zu einer Quelle die komplexe chemische Zusammensetzung des umgebenden Mediums erfasst. Die deutocerebralen chemosensorischen Loben sind aus einer Vielzahl annähernd gleichartiger Felder höherer synaptischer Dichte aufgebaut, den olfaktorischen Glomeruli. Sie sind radiär entlang der Peripherie des Neuropils angeordnet und können weiter in verschiedene strukturelle Domänen unterteilt werden. Dies wird als Ausdruck der Regionalisierung der Synapsen von lokalen Interneuronen und Projektionsneuronen verstanden (Schachtner et al. 2005).

Form und Anzahl der Glomeruli sind zwischen einzelnen, auch nah verwandten, Malacostraca sehr verschieden. So findet sich ein ganzes Spektrum von sphärisch bis länglich keilförmig, sowie von einem knappen Duzend bis weit über 1.000. Trotz verschiedener Bemühungen konnte eine phylogenetische Bedeutung dieses Glomerulikomplexes bislang nicht belegt werden. Eine Studie von Beltz et al. (2003) kommt jedoch zu dem Schluss, dass es durchaus ökologische Relevanz besitzt. Ausgehend von den untersuchten Arten, lassen sich Korrelationen von Form, Größe und Anzahl, sowie Lebensformtypen finden. Unter der Annahme, dass alle Neurite, die in einem Glomerulus terminieren die gleiche Information tragen, ein Duft also durch einen Glomerulus kodiert wird, kann die Anzahl der Glomeruli als Ausdruck des olfaktorischen Repertoires des Tieres verstanden werden - der olfaktorischen Landschaft, die das Tier zu differenzieren vermag.

Die Projektionsneurone entsenden Dendriten ausgehend von den Glomeruli über den Projektionsneuronentrakt in das Protocerebrum. Die beiden bilateralen Trakte nähern sich in der Mitte des Gehirns einander an und gabeln sich auf. Jeweils ein Teil der Neurite führt ipsilateral weiter und vereinigt sich über ein Chiasma des Traktes mit dem kontralateralen Strang, der letztendlich in unterschiedlicher Ausprägung im Komplex der Medulla Terminalis und des Hemiellipsoidkörpers mündet. Verschiedene morphologische und ethologische Befunde, vorrangig an der homologen Struktur der Hexapoda gewonnen, legen nahe, dass es sich hierbei um ein Zentrum für multimodale Integration von Stimuli handelt, das für Lernen und olfaktorische Erinnerungen von Bedeutung ist (Schmidt und Mellon 2011; Schmidt 2015).

Der zweite Pfad wird als dezentralisierte / verteilte Chemorezeption (*distributed chemoreception*) bezeichnet und wird durch bimodale Sensillen vermittelt, die, nebst den Antennen, auf der gesamten Körperoberfläche verteilt vorzufinden sind. Über diesen Pfad werden einige wenige spezifische Bestandteile des Duftes (wie bestimmte Aminosäuren) quantitativ in einem somatotopischen Kontext abgebildet. Dies ermöglicht die Lokalisierung der Quelle. Das Verständnis dieses Pfades ergibt sich maßgeblich aus Studien zur Physiologie der Sensillen sowie zum Verhalten der Tiere, eine Tiefe wie sie bezüglich der Olfaktion erreicht wurde, ist jedoch nicht gegeben. Insbesondere das Zusammenspiel der Afferenzen, d.h. wie chemosensorische und mechanosensorische Informationen integriert werden und in entsprechendem Verhalten münden, muss daher als Blackbox betrachtet werden.

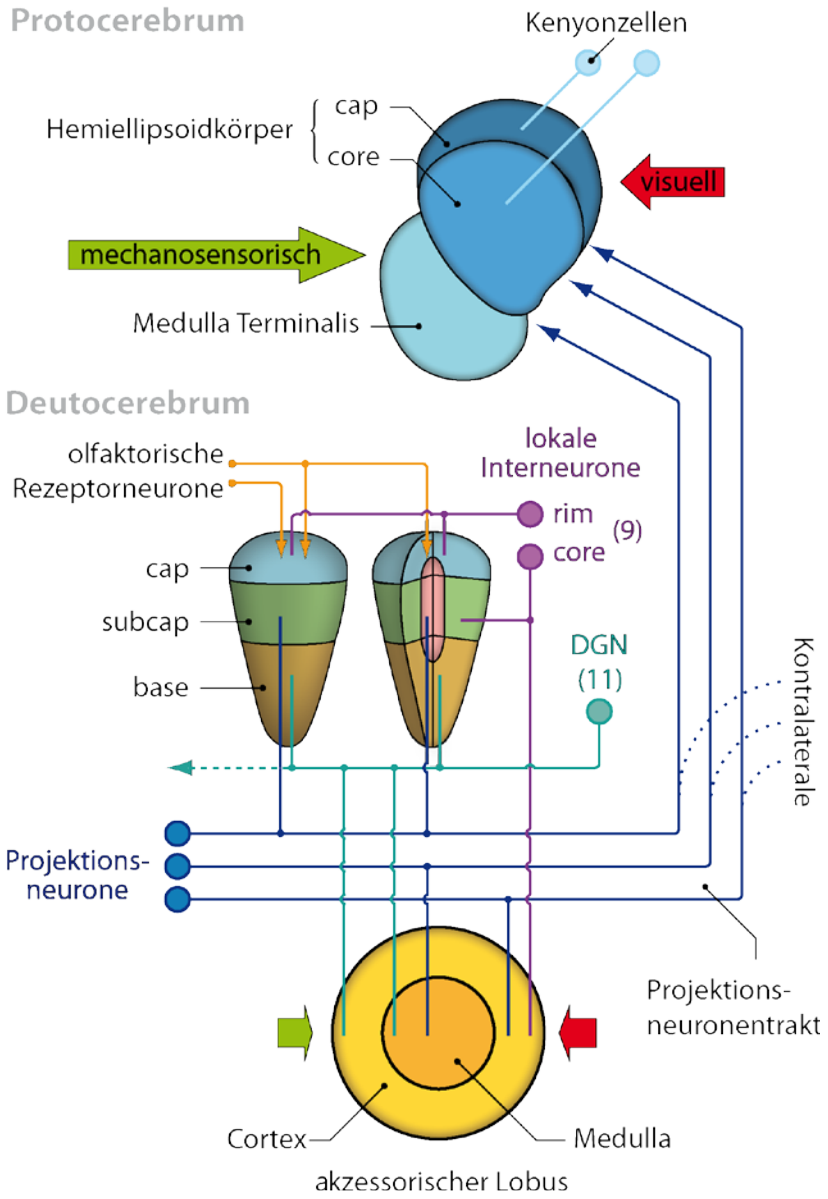

Abbildung 2.2 | Verschaltung der primären und sekundären Afferenzen im Deutocerebrum eines decapoden Malacostracen.
Die in der ersten Antenne lokalisierten olfaktorischen Rezeptorneurone projizieren über den Nerv der ersten Antenne in die olfaktorischen Glomeruli. In diesen werden die primären Afferenzen auf eine Reihe lokaler olfaktorischer Interneurone (Cluster 9 und 11, **DGN** dorsales Riesenneuron), sowie Projektionsneurone verschaltet. Zusammen mit weiteren sekundären Afferenzen aus den akzessorischen Loben (dies betrifft nur Vertreter der Reptantia, Decapoda) werden über den Projektionsneuronentrakt die prozessierten Informationen in ein multimodales Integrationszentrum projiziert - der Hemiellipsoidkörper/ Medulla-Terminalis-Komplex. Verändert nach Loesel et al. (2013).

2.2 Variationen des Themas – die Isopoda

2.2.1 Periphere Sensorische Pfade von *Saduria entomon*

Der grundlegende Aufbau der primären Riechorgane unterscheidet sich innerhalb der Crustaceen nur wenig und soll anhand der Aesthetasken von *Saduria entomon* und *Idotea balthica* erläutert werden. Diese Tiere verfügen zwischen etwa 20 (*I. balthica*; Guse 1983) und 40 - 60 (*S. entomon*; Pynnönen 1985; Kenning und Harzsch 2013) Aesthetasken (aber Vergleich Beltz et al. 2003). Diese schlanken und mehrere hundert Mikrometer messenden Sinneshaare sind im Gegensatz zu den meisten anderen Crustaceen auf das distale Ende des Flagellums beschränkt. Sie sind von einer Cuticula überzogen, die in verschiedenartiger Ausprägung sehr dünn sein kann und vor allem im distalen Bereich im Querschnitt eine radiär angeordnete Streifung aufweist. Derart werden Permeabilitätsbrücken geschaffen, die nicht selten in Poren münden (siehe hierzu Pynnönen 1985). Ferner finden sich entlang der Längsachse die Oberfläche vergrößernde cuticuläre Kämme. Die Somata der olfaktorischen Rezeptorneurone liegen direkt unterhalb der antennalen Cuticula an der Basis der Sensillen.

In *I. balthica* entsenden zwischen 60-80 Neurone ihre Dendriten in das Aesthetaskenlumen. Diese verästeln sich wiederum zu weiteren dendritischen Ausläufern. Verlässliche Zahlen gibt es diesbezüglich nicht, doch scheinen nach den Beschreibungen und Abbildungen von Guse (1983) sowie Pynnönen (1985) die dendritischen Segmente der untersuchten Isopoden weniger dicht gepackt als in den Decapoda (bspw. Snow 1973; Spencer und Linberg 1986; Hallberg und Skog 2011), was in Kombination mit der relativ geringen Anzahl an Aesthetasken als eine geringere chemorezeptive Auflösung gedeutet werden kann.

Terrestrische Isopoden stellen jedoch eine geradezu drastische Ausnahme dieses Schemas dar und sollen in Abschnitt 2.2.3 eingehender betrachtet werden. Aufbau und Bedeutung der zweiten Antenne sowie assoziierter Sensillen wurden bislang wenig berücksichtigt, entsprechende Informationen sind demnach rar. Ausgehend von Befunden zu den Decapoda kann angenommen werden, dass eine Vielzahl der Sensillen mechanosensorisch und/oder bimodal chemo-mechanosensorisch ist. Isopoden verfügen an der Spitze der zweiten Antenne jedoch über eine Kondensation von größeren, morphologisch distinkten Sensillen.

In *S. entomon* besteht diese Struktur aus einer kondensierten Ansammlung von etwa 20 - 30 Sensillen und akzessorischen Haaren, dem apikalen sensorischen Büschel (*apical sensory tuft sensu* Kenning und Harzsch 2013, in Anlehnung an Mead 1976 und Seelinger 1977). Morphologische und ethologische Befunde dieser Struktur sind lediglich für einige verschiedene Oniscidea verfügbar. In diesen konnte aber eine kontaktchemosensorische (Henke 1960; Gupta 1962; Linsenmair 1972), möglicherweise auch olfaktorische Funktion gezeigt werden (Zimmer et al. 1996).

2.2.2 Das Gehirn und die zentralen sensorischen Pfade von *Saduria entomon*

Der Aufbau des Gehirns im Allgemeinen sowie der sensorischen Pfade im Speziellen unterscheidet sich in den bislang untersuchten marinen Isopoden nur unwesentlich von dem anderer Malacostraca. Es findet sich aber auch eine Reihe an Unterschieden, die für das Verständnis der chemischen Ökologie des Tieres bedeutsam sind.

Isopoden besitzen wie Amphipoden im Unterschied zu den meisten anderen Peracariden ungestielte, sessile Augen. Die assoziierten Neuropile der Lamina, Medulla und Lobula sind daher mit in die Kopfkapsel eingegliedert und über sehr lange optische Nerven mit der Retina verbunden. Die Präsenz eines vierten Neuropils, der Lobulaplatte, konnte erst relativ kürzlich für *I. balthica* (Sinakevitch et al. 2003) sowie für *S. entomon* (Kenning und Harzsch 2013) belegt werden. Unklar ist jedoch die Natur der Verschaltung der Neuropile untereinander.

Im weiteren lateralen Protocerebrum lassen sich die Medulla Terminalis und der Hemiellipsoidkörper nur grob differenzieren. Eine Schichtung oder Unterteilung des Hemiellipsoidkörpers durch parallel verlaufende Neurite in *cap* und *core* Neuropile wie er innerhalb der Decapoda häufig beobachtet werden kann, ist in den Isopoden nicht, bzw. nur sehr schwach ausgeprägt. Lediglich im anteriomedianen Bereich kann eine höhere synaptische Dichte nachgewiesen, und dieser daher vorsichtig als *cap* angesprochen werden. Stemme et al. (2014) konnten zudem mittels antennaler Backfills eine Aufspaltung des Projektionsneuronentraktes im lateralen Protocerebrum nachweisen, die auf eine differenzielle Innervation der Medulla Terminalis und des Hemiellipsoidkörpers hindeutet. Allen bislang untersuchten Isopoden gemein ist die Aufspaltung der assoziierten Kenyon-Zellcluster (Cluster 5) in drei distinkte Auswölbungen, die dem lateralen Protocerebrum anteriodorsal aufsitzen (Harzsch et al. 2011; Kenning und Harzsch 2013; Stemme et al. 2014).

Die deutocerebralen chemosensorischen Loben stellen sich als deutliche, aber unregelmäßig geformte laterale Auswölbungen des Deutocerebrums dar. Zudem kann in *I. balthica* und *S. entomon* eine Unterteilung des Lobus in zwei Unterloben beobachtet werden. Inwiefern es sich hierbei um eine funktionelle Untergliederung handelt, ist nicht bekannt. Ein Sexualdimorphismus wie er bei einigen anderen Crustaceen oder Hexapoden auftritt, kann jedoch ausgeschlossen werden (Johansson und Hallberg 1992; Kondoh et al. 2003; Strausfeld und Reisenman 2009; El Jundi et al. 2009). Die Loben sind grundmusternah aus etwa 40 bis 80 unregelmäßig sphärischen Glomeruli aufgebaut, die entlang der Peripherie des Neuropils einen weitestgehend synapsenfreien Raum umschließen. Sie erhalten primäre Afferenzen über den Nerv der ersten Antenne von der Peripherie (Vergleich Abb. 2.2). Die olfaktorischen Glomeruli der bislang untersuchten Spezies lassen sich über ihr Neurotransmitter- und Neuropeptid-Repertoire weiter in unterschiedliche Domänen differenzieren. Von distal sind dies die *cap* und die sich daran anschließende *base*. Diese Unterteilung beruht nach Schachtner et al. (2005) auf unterschiedlichen Innervationsregionen der lokalen olfaktorischen Inter- sowie Projektionsneurone (Abbildung 2.2).

Weitere mit der ersten Antenne assoziierte Neuropile lassen sich nicht eindeutig ansprechen. Das laterale Antenne-1-Neuropil kann über den ungefähren Verlauf der Afferenzen grob umrissen werden, entbehrt aber einer offensichtlichen Strukturierung. Ob ein medianes Antenne-1-Neuropil existiert, konnte bislang nicht genügend belegt werden. Das Neuropil der zweiten Antenne ist dagegen eine prominente Auswölbung direkt anteriolateral der oesophagealen Konnektive. In *I. balthica* weist dieses Neuropil eine Strukturierung auf, die der Organisation des entsprechenden Neuropils in den Decapoda sehr ähnlich ist. Ausgehend von einem zentralen Neuritenbündel projizieren transversal abgehende Neurite in das Neuropil. Es entsteht der Eindruck eines wiederkehrenden quasi-segmentalen Musters (Harzsch et al. 2011). Diese Organisation wird als eine Karte verstanden auf welcher die Sensillen der zweiten Antenne somatotopisch abgebildet werden (Zeil et al. 1985; Schmidt 2007). Ob eine ähnliche Strukturierung auch in *S. entomon* vorhanden ist lässt sich anhand der Daten nicht endgültig entscheiden. So konnte zumindest keine derartige Organisation dargestellt werden. Dagegen findet sich aber eine für marine Crustaceen ungewöhnliche Differenzierung des Neuropils in eine Vielzahl von Mikroglomeruli (Kenning und Harzsch 2013). Da Glomeruli sich vielfach in Neuropilen finden lassen, die primär oder sekundär mit der Verarbeitung chemosensorischer Information assoziiert sind (Zusammenfassungen zur Konvergenz chemosensorischer Systeme finden sich in Strausfeld und Hildebrand 1999 und Eisthen 2002), liegt die Vermutung nahe, dass der zweiten Antenne mariner Isopoden eine wesentlich größere Bedeutung in der Wahrnehmung chemischer Stimuli zukommt, als bislang für die Malacostraca vermutet.

2.2.3 Die Oniscidea - periphere und zentrale chemosensorische Pfade

Terrestrische Isopoden zeichnen sich durch eine Reihe an sensorischen Besonderheiten aus. Zum einen sind dies die dreispitzigen Sensillen (*tricorn sensilla*) die zahlreich über die gesamte Körperoberfläche verteilt sind und als Hygro- und Thermorezeptoren interpretiert werden (Ziegler und Altner 1995). Die drastischste Modifikation jedoch betrifft die erste Antenne. Diese wurde im Verlauf ihrer Entwicklungsgeschichte sowohl erheblich verkleinert, als auch durch eine Reduktion der Antennomere auf zwei pedunkuläre und ein flagelläres Glied verkürzt (Schmalfuss 1998). Damit einhergehend verloren diese Tiere auch einen Großteil des entsprechenden Sensillenbesatzes.

In allen bislang untersuchten Oniscidea zeigt sich, dass diese Reduktion einen geradezu kaskadierenden Effekt auf alle mit der ersten Antenne assoziierten Bereiche des Gehirnes hat. In *Porcellio scaber* ist noch ein *quasi-neuropilärer,* lateraler Auswuchs des Deutocerebrums vorzufinden (Harzsch et al. 2011). Ebenso zeigt sich im immunhistochemischen Präparat eine erhöhte synaptische Dichte. Eine strukturelle Organisation auf dem Niveau wie es in anderen Crustaceen für einen deutocerebralen chemosensorischen Lobus charakteristisch ist, findet sich hier jedoch nicht. Noch deutlicher stellt sich die Situation in der Wüstenassel *Hemilepistus reaumuri* oder der Rollassel *Armadillidium vulgare* dar. Das Deutocerebrum kann hier lediglich über die ungefähre Lage innerhalb des Syncerebrum angesprochen werden, deutocerebrale chemosensorische Loben, sowie laterales und medianes Antenne-1-Neuropil, können in dem Neuropilkomplex nicht identifiziert werden (Harzsch et. al 2011). Die geringe Größe der ersten Antenne und damit ihre drastisch eingeschränkte sensorische Spannweite, sowie das Fehlen assoziierter Gehirnareale legen die Annahme nahe, dass terrestrische Isopoden ihren (antennularen) Geruchssinn verloren, oder aber zumindest stark zurückentwickelt haben. Gänzlich ohne Funktion ist die erste Antenne in den Oniscidea jedoch sicherlich nicht. An der Spitze des letzten Antennengliedes findet sich noch eine geringe Anzahl von etwa 10 - 20 zapfenähnlichen Sensillen, die durch lediglich zwei Rezeptorneuronen innerviert werden (Ábrahám und Wolsky 1930; Alexander 1977; Risler 1977; Haug und Altner 1984; Schmalfuss 1998). Diesen wird, ausgehend von physiologischen und morphologischen Befunden, eine chemosensorische Rolle im Sinne eines Luftfeuchtigkeitsdetektors zugeschrieben (Haug und Altner 1984). Eine weitere Besonderheit betrifft die zweite Antenne. Wie bei allen Malacostraca gilt diese aufgrund der Fülle an mechano- sowie bimodalen chemo- und mechanosensorischen Sensillen als der maßgebliche mechanosensorische Anhang der Isopoda (Ábrahám und Wolsky 1930; Seelinger 1977; Seelinger 1983; Wägele 1992). Ähnlich der Situation wie oben für *S. entomon* beschrieben, findet sich bei terrestrischen Isopoden an der Spitze ein Sensillenkomplex, der hier aus etwa 50 Einzelsensillen besteht - der Antennenendzapfen (*apical sensory cone*, Seelinger 1977; Mead et al. 1976). Diese Sensillen, die lediglich im terminalen Bereich frei, ansonsten aber zu einem einzigen apikalen Konus verschmolzen sind, werden jeweils von fünf bis zwölf Neuronen innerviert und tragen eine terminale Pore (Mead et al. 1976). Es konnte gezeigt werden, dass die Tiere mittels des Endzapfens auf volatile Substanzen reagieren. Sowohl Verhaltensbeobachtungen als auch Untersuchungen zur Feinstruktur legen jedoch eine mechanosensorische, vor allem aber kontakt-chemorezeptive Funktion der Struktur nahe (Mead et al. 1976; Seelinger 1977; Seelinger 1983; Wägele 1992). Insbesondere beim Suchlauf wird die zweite Antenne beständig über den Boden geführt und dieser mit einer hohen Frequenz mittels des Endzapfens beprobt (Hoese und Schneider 1990). Entsprechende Hinweise auf eine chemosensorische Funktion ergeben sich auch aus der Neuroanatomie. Harzsch et al. (2011) konnten zeigen, dass das mit der zweiten Antenne assoziierte Neuropil in *H. reaumuri*, gleich der Situation bei *S. entomon*, aus einer Vielzahl mikroglomerulärer Untereinheiten aufgebaut ist. Fraglich ist allerdings die Situation in anderen Vertretern der Oniscidea, hier fehlt bislang der Nachweis einer derartigen Kompartimentierung dieses Neuropils.

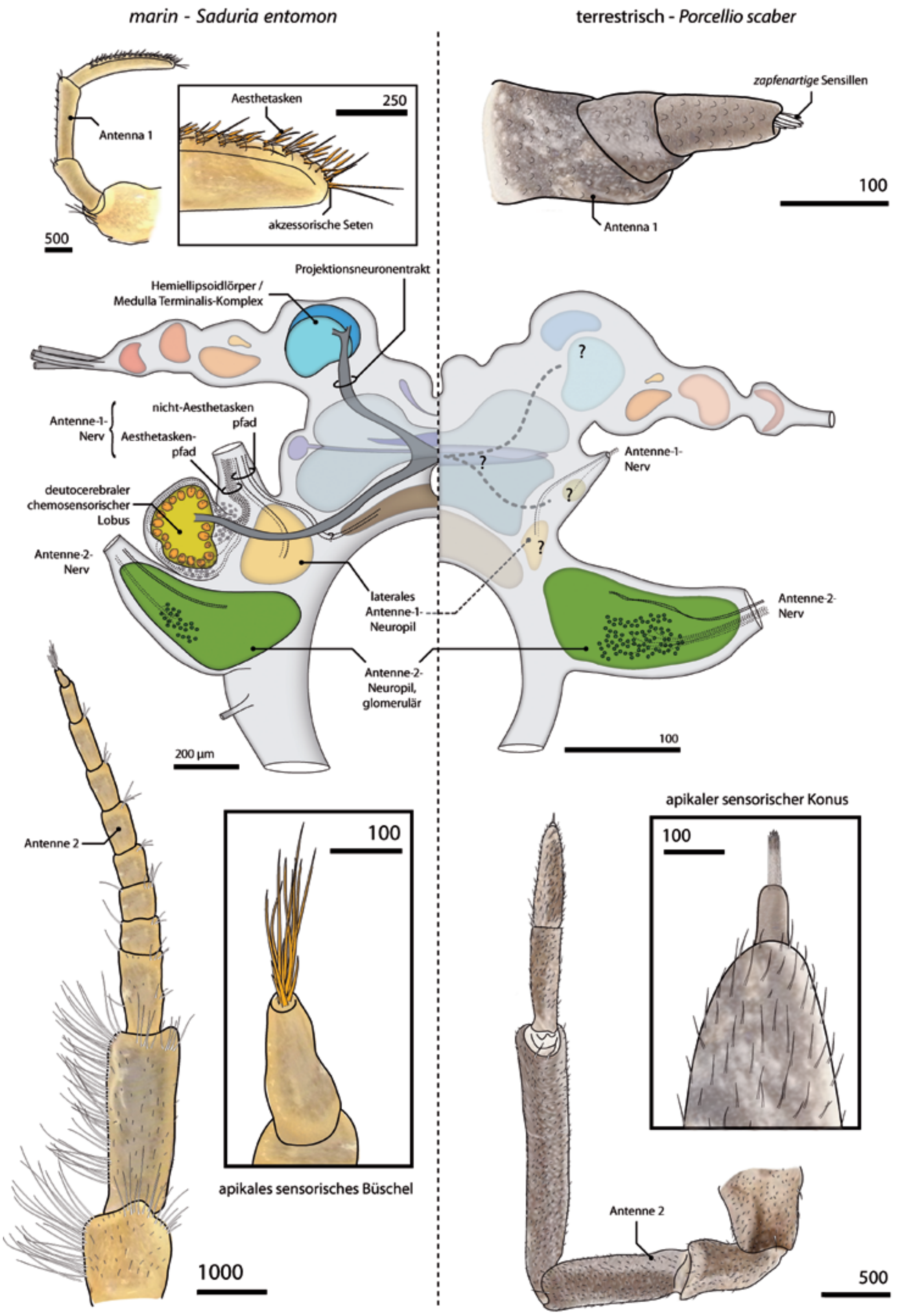

Abbildung 2.3 | Vergleich der peripheren und zentralen chemosensorischen Pfade mariner und terrestrischer Isopoden

Links. mariner Isopode am Beispiel von *Saduria entomon* Ausgehend von der ersten Antenne projizieren chemosensorischen Pfade in das Deutocerebrum. Der Aesthetaskenpfad mündet in den deutocerebralen chemosensorischen Loben, der Nicht-Aesthetaskenpfad im lateralen Antenne-1-Neuropil. Die mechanosensorischen Sensillen der zweiten Antenne, sowie die Rezeptorneurone der chemosensorischen und bimodalen Sensillen des apikalen sensorischen Büschels projizieren in das golmeruläre Antenne-2-Neuropil. **Rechts. terrestrischer Isopode am Beispiel von *Porcellio scaber*** Im Verlauf der Größenreduktion der ersten Antenne wurde gleichsam das mit dieser assoziierte neuronale Substrat reduziert. An der Spitze der ersten Antenne verfügen die Tiere noch über einige wenige Sensillen. Welcher Art diese sind und wo ihre Rezeptorneuronen terminieren ist unbekannt. Die Rezeptorneurone der chemosensorischen und bimodalen Sensillen des apikalen sensorischen Konus terminieren im glomerulären Antenne-2-Neuropil.

3. Ethologie

3.1 Geboren um zu riechen?

Neben der Morphologie und Physiologie peripherer und zentraler chemosensorischer Pfade waren auch die durch diese Pfade vermittelten Verhaltensweisen, die Phänomenologie der Chemorezeption, Gegenstand von Untersuchungen in zahlreichen Vertretern der Malacostraca. Analog der Situation zur grundlegenden sensorischen Morphologie verdanken wir unser Verständnis chemosensorisch geführten Verhaltens, neben den Insekten, auch hier lediglich einigen wenigen decapoden Krebsen (Hay 2011). Die wenigen Studien, die sich mit dem Verhalten weiterer Malacostraca, oder speziell der Peracariden beschäftigten, gewähren nur ein grobes Bild eines der Olfaktion zumindest prinzipiell befähigten Organismus. Wie dieses Verhalten sich jedoch in dieser Tiergruppe im Einzelnen, und spezieller, in kleineren zeitlichen und räumlichen Skalen darstellt, vor allem aber welchen Einfluss unterschiedliche Lebensweisen haben, ist unbekannt.
Insbesondere vor dem Hintergrund der durch die morphologischen Befunde naheliegenden „olfaktorischen Unfähigkeit“ terrestrischer Isopoden sind Untersuchungen zum chemosensorischen Potential weiterer Peracarida, respektive Isopoda, so wertvoll. Während es zum Verhalten der Oniscidea zumindest einige grundlegende Beobachtungen, jedoch nur wenige repräsentative Untersuchungen gibt, beruht unser Verständnis olfaktorischer Verhaltensweisen mariner Isopoden lediglich auf vereinzelten Beobachtungen, Annahmen und Deduktionen.

3.2 Untersuchung zum grundlegenden Geruchsunterscheidungsvermögen

Erste detaillierte Untersuchungen zum olfaktorisch gesteuerten Verhalten von *Saduria entomon* durch Kenning et al. (2015) machten deutlich, dass eine Übertragung unseres Verständnis, vor allem aber die experimentelle Methodik, auf andere Arten der Malacostraca nicht ohne weiteres möglich ist. In dieser Studie kam das klassische Konzept eines Y-Labyrinthes (Olfaktometer) zum Einsatz, um die Reaktionen von *S. entomon* auf verschiedene Düfte zu testen (Abb. 3.1 A). Maßgeblich wurden Gerüche verwendet, denen das Tier auch in seiner natürlichen Umgebung begegnen mag und die zumindest theoretisch attraktiv sein sollten, d.h. toter Fisch (Stücke oder Homogenate von Scholle, *Pleuronectes platessa* oder Hering, *Clupea harengus*), Muscheln (*Macoma balthica*, tot und geöffnet) oder Artgenossen (in Gruppen oder an Fisch nagend). Zudem wurde die Reaktion auf den Duft toter Artgenossen untersucht, obgleich hier nicht klar war, inwiefern dies eine abschreckende Wirkung im Sinne eines Kairomons hatte, oder aber ob der Karnivorie des Tieres ebenfalls attraktiv wirken könnte. Letztendlich wurde auch eine Aminosäurenmixtur getestet, die in seiner Zusammensetzung die Absonderungen von verwesendem Fisch imitiert (Ide et al. 2006). Um das gesamte experimentelle Design auf seine Praxistauglichkeit zu testen, wurden zusätzlich Experimente mit dem Marmorkrebs (*Procambarus fallax* forma *virginalis*) durchgeführt und dessen Reaktion auf den Duft eines wassergelösten Extraktes einer Futtertablette protokolliert. Alle Versuchsreihen wurden bei beiden Tieren in Dunkelheit durchgeführt um sicher zu stellen, dass lediglich chemische und hydromechanische Informationen zur Orientierung dienten. Der Marmorkrebs lokalisierte den jeweiligen Stimulus-Arm des Aufbaus in mehr als 82 % der Experimente und demonstrierte einmal mehr, mit welcher Präzision diese Tiere Duftspuren verfolgen, ihre Quelle identifizieren und lokalisieren können.

In 15 verschiedenen Experimenten, die 1,800 Einzelversuche umfassten, konnten in *S. entomon* dagegen lediglich vier Stimuli identifiziert werden, die statistisch signifikante Ergebnisse hervorbrachten. Interessanterweise und gänzlich entgegen den Erwartungen reagierte das Tier dabei auf drei der Düfte (Aggregationen von an Fisch fressenden Artgenossen, Homogenat von Hering und die Aminosäure Taurin) aversiv. Lediglich für die Aminosäure Threonin ließ sich eine attraktive Wirkung zeigen.

Eine Reihe einander nicht notwendigerweise ausschließende Ursachen können für diese Ergebnisse als Erklärung herangezogen werden. In ökoethologisch aussagekräftigen Experimenten müssen die verwendeten Stimuli in einer Form appliziert werden, die möglichst den Bedingungen im natürlichen Habitat entsprechen. Dies betrifft die Qualität und Quantität, vor allem aber die Art und Weise der Applikation. Wesentliche Eckpunkte stellen für Letzteres, wie vielfach für andere Crustaceen gezeigt, das Licht- und Strömungsregime dar (Weissburg und Zimmer-Faust 1994; Weissburg 2003; Keller und Weissburg 2004).

Ein weiterer Erklärungsansatz besteht in der Möglichkeit, dass ein Großteil der verwendeten Stimuli und/oder Konzentrationen nicht als für *S. entomon* relevant zu betrachten sind. Nicht zu unterschätzende Bedeutung kommt dem eigentlichen Versuchsaufbau zu (Vergleich Abb. 3.1). So könnten Umgebungen und Bedingungen, die zeitlich und räumlich restriktiv sind wie im Experiment angewendet, eine ökologisch wenig relevante Situation darstellen und daher eine erfolgreiche Verfolgung der Duftspur erschweren oder sogar verhindern. Es gibt jedoch noch eine mögliche Ursache, die ganz fundamental die Natur des Experimentes, respektive des Versuchstieres betrifft. So könnten duftgeführte Orientations- und Suchbewegungen nicht Bestandteil der Nahrungssuchstrategie von *S. entomon* sein, und Distanzchemorezeption daher über längere Strecken, zumindest zum Zwecke des Nahrungserwerbs, nicht relevant. *Olfaktion* diente daher, im Sinne der eigentlichen Definition, lediglich einer Qualitätsbeurteilung.

Es drängt sich dann jedoch die Frage auf, wie das Tier überhaupt in der Lage ist geeignete Nahrung zu finden. Zum einen könnte sich *S. entomon* auf einen glücklichen Zufall verlassen. Dies ist ökophysiologisch wie evolutionär schwerlich nachzuvollziehen und reflektiert wohl lediglich den Mangel an geeigneten Tests.

Verschiedene Berichte von Forschungstauchern als auch eine Anmerkung von Narver (1968), sowie Aufnahmen aus dem Habitat von *Saduria*'s Pfaden im Schlick, deuten jedoch tatsächlich eine gewisse Wahllosigkeit in der Bewegung des Tieres an, und mehren Zweifel an seinen chemotaktischen Fähigkeiten.

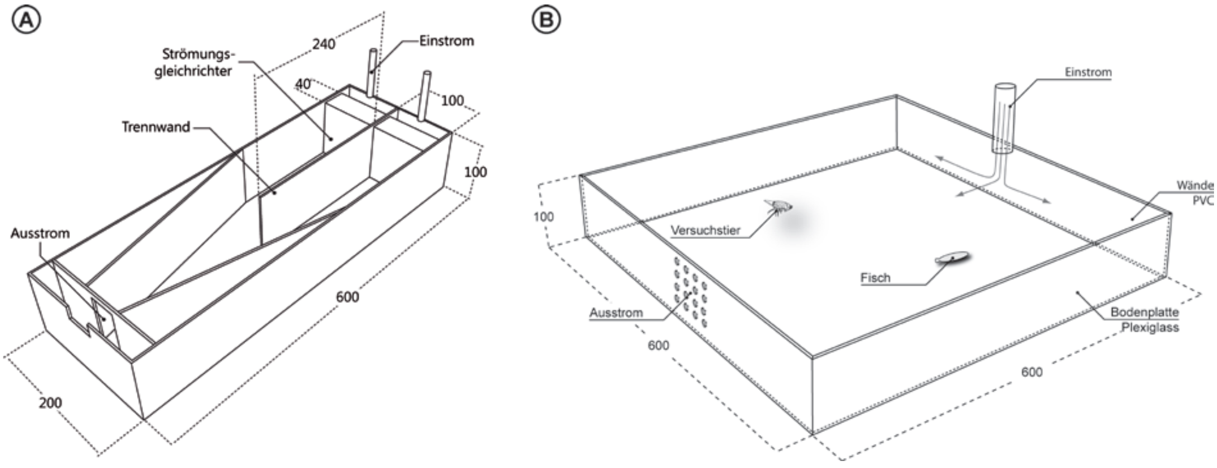

Abbildung 3.1 | Versuchsaufbauten für die Untersuchung chemosensorisch geführten Verhaltens in Crustaceen

A Y-Labyrinth *alias* Olfaktometer für die Untersuchung des grundlegenden Geruchsunterscheidungsvermögens. Die Applikation eines im Wasser gelösten Stimulus erfolgt in einer der beiden Einströmöffnungen. Das Versuchstier akklimatisiert an der Ausströmöffnung. In Abhängigkeit der Fließgeschwindigkeit wird das Tier in das Labyrinth entlassen, sobald die Duftfahne den Ausstrom erreicht. Die Reaktionen und Entscheidungen des Tieres werden protokolliert. Abmessungen in Millimeter. Verändert nach Kenning et al. 2015. **B Die Arena**. Versuchsaufbau zur Untersuchung von Suchstrategien. Ein Stimulus wird zufällig in der Arena platziert, das Versuchstier akklimatisiert am Ausstromende. Die Bewegungen sowie der Sucherfolg des Tieres werden aufgezeichnet und evaluiert. Abmessungen in Millimeter.

3.3 Untersuchung zu Suchstrategien

Um sowohl die Frage zu beantworten wie *S. entomon* in der Lage ist Nahrung zu finden, als auch den Aspekt der räumlichen Orientierung mit in Betracht zu ziehen, wurde von Kenning et al. (*eingereicht*) ein Experiment konzipiert, in welchem *S. entomon* in einer quadratischen Durchflussarena von 3,600 cm^2 Fläche in verschiedenen experimentellen Designs in Dunkelheit einen Stimulus (Stint, *Osmerlus eperlanus*) ausfindig machen sollte (Abb. 3.1 B). Ausgehend von den Ergebnissen der ersten Untersuchung zum olfaktorischen Potential von *S. entomon* ging es zunächst um die Frage, inwiefern für das Nahrungssuchverhalten eine Unterscheidung zwischen Chemotaxie und Chemokinesie getroffen werden muss.

Taxie basiert auf der Bestimmung einer Gradientenrichtung, d.h. die Richtung der größten lokalen Ab- oder Zunahme der Stimulusintensität, sei diese visueller, mechanischer oder chemischer Natur. Diese Richtung stimmt im Idealfall mit der des Stimulus und letztendlich mit der eingeschlagenen Bewegungsrichtung überein (Bovet und Benhamou 1990; Doucet und Dunn 1990). Die wahrgenommene Gradientenrichtung ist jedoch naturgemäß zahlreichen Schwankungen und Ablenkungen unterworfen, die aus Fluktuationen des Signals (durch z.B. Turbulenzen des Mediums) entstehen oder aber entsprechend der Modalität der empfangenen Information eine geringere Präzision zulassen (bspw. erlaubt Sehen in den meisten Fällen eine exaktere Lokalisation als Chemorezeption). Zwar haben beide Fälle eine geringere Sucheffizienz zur Folge, doch wird letztendlich die Drehbewegung des Tieres eine Tendenz zum Stimulus hin oder von diesem weg aufweisen.

Im Gegensatz dazu beruht Kinesie nicht auf der Bestimmung der absoluten Gradientenrichtung, sondern wird als eine Abwägung der Variationen des Potentials, während sich das Tier durch den Gradienten hindurchbewegt, verstanden. Während die Grundlage für chemotaktisches Verhalten also eine Veränderung der tatsächlichen Drehrichtung darstellt, wird in der chemokinetischen Orientierung die Frequenz und das Ausmaß der Drehrichtung moduliert (Fraenkel und Gunn 1961; Benhamou und Bovet 1992).

Pynnönen (1985) führte bereits erste Ablationsversuche der Aesthetasken von *S. entomon* durch und konstatierte, dass diese zwingend notwendig für olfaktorische Orientierungsreaktionen seien. Um die Bedeutung der Aesthetasken beim Suchlauf zu klären, wurden in diesem Experiment einigen Tieren daher die ersten Antennen durch Immersion in destilliertem Wasser chemisch ablatiert. Ebenso wurden bei einigen Tieren die zweiten Antennen in gleicher Weise inaktiviert, um die vermutete chemosensorische Funktion dieser zu testen.

Die Bewegungen von *S. entomon* in der Arena wurden mittels einer infrarotsensitiven Digitalkamera dokumentiert. Eine Reihe von Bewegungsparametern, wie Geschwindigkeit, der oben beschriebene Drehwinkel oder der Navigationsfehler (d.h. die Abweichung von Bewegungs- zu Stimulusrichtung) wurden erhoben und für die Evaluation der Suchstrategien herangezogen.

Ausgehend von den Bewegungsmustern der Kontrolle, die als Modell einer weitestgehend ungestörten Bewegung verstanden werden kann, zeigten sich deutliche Veränderungen in den beobachteten Bewegungsparametern, wenn ein Stimulus in die Arena eingebracht wurde. Dieser wurde daher, wie schon durch Pynnönen (1985) angenommen, mit ziemlicher Sicherheit aus der Distanz wahrgenommen.

Ein bei anderen Crustaceen häufig beobachtetes Verhalten, das die Rezeption eines Duftes anzeigt, ist das sogenannte *antennal flicking*. Dies bezeichnet kurze, schnelle, ruckartige Bewegungen der ersten Antennen, die als räumlich und zeitlich hochaufgelöstes Schnüffeln interpretiert werden. Die Bewegung führt zu einem gesteigerten Wasseraustausch an den antennalen Rezeptoren und verbessert derart die Duftwahrnehmung (Koehl 2011). Dieses Verhalten, das lediglich für wenige weitere Peracariden beschrieben ist (bspw. für Amphipoden, Kaufmann 1994), wurde in den vorliegenden Verhaltensuntersuchungen jedoch nicht beobachtet. Allerdings nutzt *S. entomon* gelegentlich die ersten Peraeopoden um das erste Antennenpaar abzustreifen. Bereits Pynnönen (1985) beschreibt dieses Verhalten, das jedoch nicht auf die erste Antenne beschränkt ist, sondern auch mehrfach an der zweiten Antenne beobachtet werden konnte. Inwieweit diesem Verhalten eine Funktion im Sinne des *flicking* zugeschrieben werden kann, ist daher fraglich. Vielmehr dürfte es als ein weiteres Indiz dafür angesehen werden, dass das olfaktorische System von *S. entomon* mit einer geringeren räumlichen und/oder zeitlichen Auflösung operiert.

Im Gegensatz zu den Untersuchungen von Kenning et al. (2015) war *S. entomon* in diesem Experiment mit einer Erfolgsquote von 90 % in der Lage, nach einer gewissen Zeit den Stimulus zu lokalisieren. Eine Stimulus-Gerichtetheit der Bewegungen oder der Suchstrategie war jedoch nicht auf den ersten Blick offensichtlich, da die zurückgelegten Pfade, ähnlich zu den Beschreibungen aus dem Habitat, langgezogene und ausufernde Spiralbewegungen umfassten. Einen direkten Pfad vom Start zum Stimulus, und damit über jeden Zweifel an Chemotaxie erhaben (sehr eindrücklich dargestellt in Moore et al. 1991), gab es in keinem der Experimente.

Ebenso konnte in mehreren Einzelversuchen beobachtet werden, wie das Tier den Stimulus in einer Entfernung von nur wenigen Zentimetern, ohne eine erkennbare Reaktion zu zeigen die als „olfaktorische Auf- oder Erregung" (d.h. Stoppen, Orientierung, *flicking*; siehe bspw. Kennedy 1978) interpretiert werden könnte, passiert. Ein statistischer Orientierungstest zur Unterscheidung von Taxie und Kinesie anhand der relativen Drehwinkel (*sensu* Benhamou und Bovet 1992) kam jedoch zu dem Ergebnis, dass es sich bei den gezeigten Bewegungen tatsächlich um chemotaktische Orientierung handelt, und nicht wie die Pfade vermuten ließen, um eine chemokinetische oder bloßen Zufall. Darüber hinaus zeigte die Analyse, dass die Chemotaxie möglicherweise eine rheotaktische Bewegung überlagert. Interessanterweise konnte diese strömungsgerichtete Bewegung nur in Experimenten beobachtet werden in denen auch ein Fisch als Stimulus verwendet wurde. Hydromechanische Informationen werden demnach integrativ bei Vorhandensein eines chemischen Stimulus zusätzlich für die Orientierung genutzt.

Die Ablation der ersten Antenne führte zu einer beinahe vollständigen Unfähigkeit den Fisch ausfindig zu machen und verdeutlicht abermals wie entscheidend die Aesthetasken auch in dieser Spezies für Nahrungssuchstrategien sind. Lediglich 30% der Tiere waren nach, statistisch nicht signifikant, längerer Zeit in der Lage den Stimulus zu lokalisieren. Dass die Tiere überhaupt Erfolg hatten mag das Ergebnis der rein mathematischen Notwendigkeit sein, jeden Punkt der Arena nach einer gewissen Zeit zu besuchen. Es besteht jedoch auch die Möglichkeit, dass die Tiere mittels der noch funktionstüchtigen zweiten Antenne ausreichend Informationen über ihre Umgebung wahrnehmen konnten. Ob dies chemische, hydromechanische oder beide Informationen umfasst, kann nicht mit Sicherheit belegt werden. Der Orientierungstest kam zu dem Ergebnis, dass die Tiere bezüglich des Stimulus sich chemokinetisch, oder tatsächlich zufällig, orientiert haben. Zudem trat die schon im vorigen Versuch beobachtete Rheotaxie in diesem Experiment noch deutlicher hervor. Interessanterweise ergab sich durch die Ablation der zweiten Antenne ein sehr ähnliches Bild. Auch in diesem Experiment sind lediglich 30 % der Tiere erfolgreich gewesen, obgleich die Funktion der ersten Antenne und der auf dieser lokalisierten Aesthetasken nicht beeinträchtigt waren. Chemotaxie konnte über eine statistische Auswertung als elementarer Orientierungsmechanismus ausgeschlossen werden. Es zeigte sich aber wie schon im Experiment zuvor eine starke rheotaktische Komponente in den Bewegungen. Ob der Stimulus im Zuge dieser durch Zufall entdeckt, oder durch Chemokinesie ausfindig gemacht wurde, konnte nicht abschließend geklärt werden.

Deutlich wird durch dieses Experiment jedoch, wie bedeutsam das Zusammenspiel der chemo- und mechanosensorischen Afferenzen der ersten und zweiten Antenne von *S. entomon* für einen effizienten Suchlauf ist.

4. Synthesis

Die makroskopische, ultrastrukturelle und neuroanatomische Natur der sensorischen Pfade von *Saduria entomon* weist im Vergleich mit anderen Malacostracen einige, für das Verständnis der chemischen Ökologie der Tiere entscheidende, morphologische Abweichungen und Besonderheiten auf. So verfügen die Tiere beispielsweise nur über wenige und relativ kleine olfaktorische Glomeruli. Während die eigentliche Rolle der Glomeruli in der Kodierung eines Geruches nicht gänzlich verstanden ist (Beltz et al. 2003; Watanabe et al. 2010; Schmidt und Mellon 2011; Strausfeld 2012), kann zumindest angenommen werden, dass ihre Anzahl Ausdruck des olfaktorischen Repertoires des Tieres ist. Eine geringe Anzahl geht damit jedoch nicht zwangsläufig mit einer geringeren olfaktorischen Leistungsfähigkeit einher. So kann das System lediglich auf einige wenige, dafür möglicherweise umso spezifischere Odoranten (im Sinne der Einzelkomponenten eines Duftes) abgestimmt sein. Gleiches mag für die eigentlichen Geruchsorgane, die Aesthetasken gelten.

Einen Duft an sich wahrzunehmen und zu erkennen ist jedoch nur eine Seite der Medaille. Um ein Ziel auch effizient lokalisieren zu können, müssen aus der Duftfahne entsprechende, richtungsweisende Informationen extrahiert und verarbeitet werden. Zwei Punkte sind in diesem Zusammenhang wesentlich. Während beispielsweise einem Lichtreiz bereits eine Richtungsinformation immanent ist, tragen chemische Stimuli lediglich die Informationen *DAS* etwas riecht. *Saduria entomon* besitzt im Vergleich mit den Decapoda jedoch nur über eine relativ geringe sensorische Spannweite, d.h. einen vergleichsweise kleinen Bereich der von den ersten Antennen abgedeckt werden kann um eine Richtungsinformation aus den Konzentrationsasymmetrien herzuleiten. Weiterhin sind die Gehirnareale die, soweit von anderen Spezies bekannt, mit der Verarbeitung sowie Integration von chemischen und mechanosensorischen Informationen in einem propriotiven Kontext assoziiert sind, in *S. entomon* strukturell nur schwach ausgeprägt. Zusammengenommen spricht dies für eine untergeordnete Bedeutung des olfaktorischen Systems. Es darf jedoch nicht außer Acht gelassen werden, dass die biologischen Mechanismen, die es erlauben einen Stimulus unbekannter Lokalisation zu „detektieren", nicht automatisch die gleichen sind, wie diejenigen um diesen auch tatsächlich zu „finden". Der Prozess der Lokalisierung kann folglich auch durch gänzlich andere Mechanismen optimiert werden, als durch den Einsatz hochentwickelter und komplexer olfaktorische Systeme, deren Erhaltung in physiologischen Begriffen überdies energetisch kostspielig ist. Doch auch die Ergebnisse zum olfaktorisch geführten Verhalten, sowie dem Verhalten das diese Tiere, im Vergleich mit bspw. den Decapoda, nicht zeigen, stellen unser Verständnis der chemischen Ökologie dieses Tieres, vielleicht sogar der Valvifera insgesamt, weiterhin in Frage.

So entbehrt das Verhalten von *S. entomon* die für andere Malacostraca beschriebene Effizienz und Präzision in der Lokalisierung eines Stimulus (Vergleich bspw. Moore et al. 1991; Moore und Grills 1999; Moore und Crimaldi 2004), und legt darüber hinaus eine gewisse Zufälligkeit nahe. Auch wenn der (rein statistische) Orientierungstest zeigt, dass *S. entomon* sich zum Zwecke der Nahrungssuche einer chemotaktischen Orientierung bedient, doch darf dennoch nicht außer Acht gelassen werden, dass Taxie und Kinesie nur als die Endpunkte eines Spektrums verstanden werden sollten. So beinhalten die meisten Suchverhalten neben offenbar ziel- oder stimulusorientierten Bewegungen auch zufällige Komponenten (Bell 1990; Bartumeus et al. 2008). Welche von diesen letztendlich bestimmend in Erscheinung treten, als wie effizient also eine Kinesie respektive Taxie zu bezeichnen ist, hängt von dem sensorischen Potential des Tieres ab, d.h. von seiner Diskriminationsfähigkeit bezüglich der Qualität und Quantität, sowie davon alle verfügbaren Informationen in einen somatotopischen Kontext zu bringen.

Nun besteht kein Zweifel daran, dass *S. entomon* in der Lage ist Aminosäuren in einer Konzentration von wenigen Millimol wahrzunehmen (Kenning et al. 2015). Einige Chemorezeptoren der Antennen und Dactyli von decapoden Krebsen, aber auch Amphipoden, sprechen sogar ganz spezifisch nur auf einige wenige Aminosäuren an, und dies in Konzentrationen von wenigen Nanomol (Garm et al. 2005; Schmidt und Mellon 2011). Im Lichte der Ergebnisse der morphologischen und ethologischen Studien aber zeigt sich, dass diese Informationen in einen räumlichen Zusammenhang zu setzen und wortwörtlich wegweisende Informationen zu extrahieren, anscheinend nicht grundlegender Bestandteil von Nahrungssuchstrategien dieses Tieres ist.

Als ein möglicher, proximater Grund dafür kann die Struktur und Ökologie des Habitats in Betracht gezogen werden. In ressourcenarmen Habitaten wie den Tiefseeebenen sind fein abgestimmte und effiziente chemosensorische Systeme unabdingbar um Nahrung zu finden. Tiefseeamphipoden haben beispielsweise eine hochmotile Lebensweise entwickelt, die durch einen energiesparenden Metabolismus und effiziente Rezeptorsysteme charakterisiert ist (Premke 2003). Vorrangig Letzteres stellt eine schnelle Lokalisierung potentieller Nahrungsressourcen sicher. Für *S. entomon* besteht diese Notwendigkeit scheinbar nicht. In einigen Bereichen der nördlichen Ostsee erreicht *S. entomon* Bestandsdichten von mehr als einhundert Tieren pro Quadratmeter. Auch das Gebiet, in dem die Versuchstiere gefangen wurden, dürfte diesen Zahlen in Nichts nach stehen. Allein dies deutet darauf hin, dass an geeigneter Nahrung für *S. entomon* kein Mangel zu herrschen scheint. Diese zu lokalisieren ist theoretisch also nur eine Frage der Ausdauer. Eine ultimate Ursache ist in einem evolutiven Kontext schwerlich zweifelsfrei zu identifizieren. Basierend auf den vorhandenen Informationen zur Morphologie und Phänomenologie der Chemorezeption von *Saduria entomon* und der Oniscidea, lässt sich jedoch ein Szenario umreißen, das vor allem die Entwicklung und sensorische Signifikanz der zweiten Antenne, sowie die *olfaktorische Rückentwicklung* innerhalb der terrestrischen Isopoden betrifft. Bislang wurde davon ausgegangen, dass es entgegen ihrem Erfolg, die Landasseln nicht geschafft haben ein olfaktorisches System zu evolvieren, das den Anforderungen an Land, d.h. in der Luft zu operieren, gerecht wird. Als eine Art der Kompensation entwickelte sich *de novo* ein chemosensorisches System in welchem die zweite Antenne und ihr neuronales Substrat transformiert wurden um chemischen Reize wahrnehmen und verarbeiten zu können (*sensu* Harzsch et al., 2011). Nun finden wir uns jedoch in einer Situation wieder, in der nicht nur mindestens ein terrestrischer Isopode tritocerebrale Glomeruli besitzt, sondern ebenso mindestens ein, darüber hinaus relativ nah verwandter, mariner Vertreter. Unter der Annahme, dass sich sowohl die Befunde zur Neuroanatomie als auch zu den peripheren sensorischen Pfaden als generisch und nicht als konvergente Koinzidenz erweisen, kann hypothetisiert werden, dass entsprechende Vorstufen zu dieser strukturellen Organisation bereits im letzten gemeinsamen Vorfahren zu finden waren. Ferner bedeutet dies, dass für die Oniscidea diese Merkmale als Präadaptation verstanden werden können, die im Verlaufe ihrer Terrestrialisierung die antennulare Olfaktion zweitrangig, wenn nicht sogar obsolet machte.

Diese Ergebnisse zur chemischen Ökologie weiter zu validieren, hat unter Berücksichtigung zusätzlicher, basaler wie abgeleiteter Taxa, jedoch Gegenstand weiterführender Studien zu sein. Insbesondere Untersuchungen zu den Sphaeromatidea dürften, so die von Wetzer (2002) vorgeschlagene Phylogenie zugrunde gelegt wird, wertvolle Einblicke in die Evolution der chemosensorischen Systeme der Isopoda gewähren.

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6. Schriftenverzeichnis

Publikation I

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Brain anatomy of the marine isopod *Saduria entomon* Linnaeus, 1758 (Valvifera, Isopoda) with special emphasis on the olfactory pathway

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Brain anatomy of the marine isopod *Saduria entomon* Linnaeus, 1758 (Valvifera, Isopoda) with special emphasis on the olfactory pathway

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Representatives of at least six crustacean taxa managed to establish a terrestrial life style during their evolutionary history and the Oniscidea (Isopoda) are currently held as the most successfully terrestrialized malacostracan crustaceans. The brain architecture of terrestrial isopods is fairly well understood and studies on this field suggest that the evolutionary transition from sea to land in isopods coincided with a considerable size reduction and functional loss of their first pair of antennae and associated brain areas. This finding suggests that terrestrial isopods may have no or poor abilities to detect volatile substances but that their chemosensory ecology is most likely restricted to contact chemoreception. In this study, we explored how the brain of a marine isopod and particularly its olfactory system compares to that of terrestrial relatives. Using histochemical and immunohistochemical labeling, brightfield and confocal laser-scan microscopy, we show that in the marine isopod *Saduria entomon* aesthetascs on the first pair of antennae provide input to a well defined deutocerebrum (DC). The deutocerebral chemosensory lobes (DCL) are divided into spherical neuropil compartments, the olfactory glomeruli (og). Secondary processing areas in the lateral protocerebrum (lPC) are supplied by a thin but distinct projection neuron tract (PNT) with a contralateral connection. Hence, contrary to terrestrial Isopoda, *S. entomon* has at least the neuronal substrate to perceive and process olfactory stimuli suggesting the originally marine isopod lineage had olfactory abilities comparable to that of other malacostracan crustaceans.

Keywords: isopoda, central nervous system, immunohistochemistry, neurophylogeny, olfaction

INTRODUCTION

The Isopoda (Peracarida; **Figure 1**) comprise roughly 10,000 known species, but more cryptic biotopes like the deep sea remain to be explored for isopod diversity. The body size of isopods ranges from a few hundred micrometers in the interstitial Microcerberidea to impressive 50 cm in *Bathynomus giganteus*. In their over 300 million years lasting history, with a fossil record dating back to the Carboniferous (Bandel, 1967; Wilson, 2008), the Isopoda underwent a extensive radiation and colonized almost every aquatic habitat ranging from the deepest trench to shallow shelf waters and freshwater lakes. Besides scavengers, isopods are parasites, predators, and cannibals as well as prey, but they are also known for their highly developed social behavior (Kaestner, 1993; Schmalfuss, 2003; Duffy and Thiel, 2007; Linsenmair, 2007; Schmidt, 2008). Along with the first terrestrial ancestors of Hexapoda, at least five lineages of malacostracan crustaceans independently succeeded in colonizing land (Bliss and Mantel, 1968; Powers and Bliss, 1983; Greenaway, 1988, 1999; Hartnoll, 1988). While most of these taxa are still constrained to an aquatic milieu during larval development, several representatives of Oniscidea (e.g., the xerophilic desert ispod *Hemilepistus reaumuri*) achieved a level of terrestrialness that completely released them from their marine heritage, likely making Isopoda the most successful land living crustaceans.

The evolutionary transition from marine to terrestrial environments requires a number of physiological adaptations. These relate, for example, to gas exchange, ion and water balance, excretion, thermoregulation, molting, and reproduction (Bliss and Mantel, 1968; Edney, 1968; Powers and Bliss, 1983; Burggren and McMahon, 1988; Greenaway, 1988, 1999, 2003; McMahon and Burggren, 1988). What is more, sensory organs must function in air instead of water. This has in many cases a profound impact on the morphology and physiology of visual, mechanosensory and chemosensory systems, reshaping these organs during the

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Abbreviations: (n), cellcluster; a, anterior; A1Nv, antenna 1 nerve; A2Nv, antenna 2 nerve; ae, aesthetasc; AnN, neuropil of antenna 2; ao, apical organ; cA, "chemosensory" afferents; CB, central body; cN, coarse neuropil; d, dorsal; iCh?, presumptive inner optic chiasm; HE, hemiellipsoid body; DC, deutocerebrum; DCL, deutocerebral chemosensory lobe; DGN, dorsal giant neuron; fl, flagellum; l, lateral; La, lamina; lal, lateral accessory lobe; LAN, lateral antenna 1 neuropil; LNv, labral nerve; lF, lateral foramen; Lo, lobula; LoP, lobula plate; lPC, lateral protocerebrum; MAN?, presumptive median antenna 1 neuropil; MdC, mandibular commissure; MdNv, mandibular nerve; Me, medulla; mA, "mechanosensory" afferents; mF, median foramen; mPC, median protocerebrum; MT, medulla terminalis; oc, esophageal connective; oCh, outer optic chiasm; oe, esophageal foramen; og, olfactory glomeruli; oNv, optic nerve; optN, optic neuropils; PC, protocerebrum; PCC, protocerebral commissure; PB, protocerebral bridge; pe, peduncle; PNT, projection neuron tract; PT, protocerebral tract; R, retina; TC, tritocerebrum; TNv, tegumentary nerve; vC, visceral commissure; VNC, ventral nerve cord; W, X, Y, Z see text.

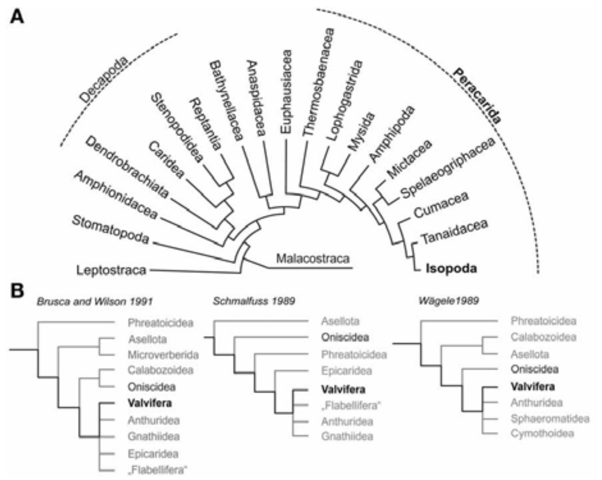

FIGURE 1 | Phylogenetic relationships of Malacostraca and Isopoda. **(A)** Phylogeny of Malacostraca compiled after Richter and Scholtz (2001) **(B)** Competing hypotheses on the phylogenetic relationships within Isopoda and unresolved positioning of Valvifera according to Brusca and Wilson (1991), Schmalfuss (1989) and Wägele (1989).

evolutionary colonization of the new habitat (Weissburg, 2010; Hansson et al., 2011; Hay, 2011). In general, our current knowledge of the organization of crustacean olfactory systems is heavily biased toward members of the Decapoda [e.g., crabs, crayfish clawed and spiny lobsters; reviewed by, e.g., Schachtner et al. (2005); Schmidt and Mellon (2011); Harzsch et al. (2012); Sandeman et al. (in press)]. However, studies that examined land living hermit crabs (*Birgus latro*: Krieger et al., 2010; *Coenobita clypeatus*: Harzsch and Hansson, 2008; Brown and Wolff, 2012; Polanska et al., 2012) point to a sophisticated and well adapted olfactory system in terms of antennular and neuronal morphology, suggesting that these animals were successful in establishing aerial olfaction (Hansson et al., 2011).

To answer the question why terrestrial isopods which have mastered crucial steps to cope with a life on land failed in adapting their olfactory system to function in air it is necessary to understand the chemosensory system of the ancestral isopod lineages that lived in the marine habitat. Therefore, this study sets out to enrich our knowledge on brain architecture in marine Isopoda by analyzing the neuroanatomy of the Baltic Sea glacial relict *Saduria entomon* (Valvifera) with special respect to the olfactory pathway. The phylogeny of Isopoda is still subject of intensive discussions and the Valvifera have been rooted in almost every position of the phylogenetic tree (Schmalfuss, 1989; Wägele, 1989; Brusca and Wilson, 1991; Wetzer, 2002; Schmidt, 2008; Richter et al., 2009; Wirkner and Richter, 2010; see **Figure 1B**) but the long-tailed morphology of *S. entomon* suggests a derived position (Brusca and Wilson, 1991). We are perfectly aware of the fact that for tracing the evolutionary transition from marine to terrestrial habitats in Isopoda, obtaining information from a more ancestral marine taxon would be preferrable. However, we are confident that the data on the brain of this *S. entomon* provided in the following is valuable in its own, and also might serve as a proxy for the plesiomorphic state of the brain architecture in marine Isopoda, nonetheless. Whereas terrestrial Isopoda have repeatedly served as models to study the olfactory pathway with respect to morphology, physiology, and behavior, our knowledge on the chemical ecology of marine isopods is close to zero [reviewed in Thiel (2011)]. Among the few studies on chemically-guided behavior in aquatic isopods that we are aware of, are those of Thompson and Manning (1981) on mate choice in the freshwater isopod *Asellus aquaticus* (Asselota) and on feeding behavior in the marine *S. entomon* (Valfivera) (Green, 1957). In the latter the antennular morphology and aesthetasc ultrastructure has been examined by Pynnönen (1985). As for behavior, the few available studies on the isopod brain architecture have focused on terrestrial representatives with the most comprehensive accounts dating back to the early 20th century (Gräber, 1933; Walker, 1935; Hanström, 1968; Alexander, 1970; Warburg and Rosenberg, 1978). Classical morphological descriptions also exist for the brains of two fully terrestrial members of the Oniscidea, *Armadillidium vulgare* (Schmitz, 1989) and *Hemilepistus reaumuri* (Kacem-Lachkar, 2000) in both of which the deutocerebral olfactory pathway has eroded away. More recently, (immuno)histochemical studies included the characterization of the optic neuropils (optN) underlying the compound eyes (Sinakevitch et al., 2003) and the localization of various neurotransmitters in the brain and ventral nerve cord (Warburg and Rosenberg, 1978; Martin and Dubois, 1981; Thompson et al., 1994; Nussbaum and Dircksen, 1995; Fouda et al., 2010; Wilcockson et al., 2011) whereas the nervous system of marine isopods remains poorly examined.

MATERIALS AND METHODS

S. entomon has a circumpolar distribution and is abundantly found in the northern Baltic Sea (Bothnian, Åland and Archipelago Seas, and in the Gulf of Finland), the Black Sea and certain boreal freshwater lakes. The animals studied here were collected in the Tvärminne Storfjärd, Gulf of Finland (Baltic Sea) near the Tvärminne Zoological Station with permission and support of the station authorities. For immunohistochemical experiments, a total of 15 specimens of both sexes were anaesthetized in ice-cooled mineral water, decapitated and fixated in either (i) 4% paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS), pH 7.4 for 2 h or (ii) for 24 h in 4% zinc-paraformaldehyde (for details see Ott, 2008) and subsequently stored in PBS (i) or HEPES buffer at 4°C (ii). Vibratome sectioning and immunohistochemical labeling of the tissues followed standard protocols (see, e.g., Harzsch et al., 2011; Sombke et al., 2011; Krieger et al., 2012; Kenning et al., 2013). Whole-mount preparations were performed according to Ott (2008). **Table 1** summarizes all labeling procedures and antibodies used. Immunohistochemical labeling was primarily used to visualize the neuronal and neuropil architecture. Thus, we applied a small range of antibodies, all of which are well established and known to label their respective antigens in the brains of arthropods. An overview of the specificity of the antibodies used can be found in Kenning et al. (2013). For histological preparations, Bouin fixated heads (saturated picrinic acid, formaldehyde, glacial acetic acid, 15:5:1) were dehydrated in an ascending series of ethanol, followed by a single incubation in xylene and two consecutive infiltrations of paraffin at 60°C for

Table 1 | Primary and secondary antibodies used in the study.

Labeling agent	Dilutions and specifications
PRIMARY	
Polyclonal rabbit anti-FMRFamide	1:2000; Acris/Immunostar; Cat. No. 20091; Harzsch et al., 2011; Krieger et al., 2012; Kenning et al., 2013
Monoclonal mouse anti-synapsin	1:30; SYNORF1, DSHB; Harzsch et al., 2011; Krieger et al., 2012; Kenning et al., 2013
Polyclonal rabbit anti-5-HT	1:1000; Immunostar, Cat. No. 20080, Kenning et al., 2013
Anti-tyronisated tubulin	1:1000; Sigma–Aldrich; Cat. No.T9028; Sombke et al., 2011; Kenning et al., 2013
SECONDARY	
Anti-rabbit AlexaFluor488	Goat anti-rabbit IgG (H + L) antibody, Invitrogen; MolecularProbes; Cat. No. A-11008
Anti-mouse Cy3	Cy3-conjugated AffiniPure goat anti-Mouse IgG (H + L) antibody, Jackson ImmunoResearch Laboratories Inc. Cat. No. 115-165-003
NUCLEAR COUNTER STAIN	
	0.05%, bisBenzimid H 33258, Sigma–Aldrich; Cat. No. 23491-45-4

1 and 2 h, respectively. The specimens were then removed and embedded in fresh paraffin. Horizontal sections were cut at a thickness of 6 μm using a microtome (Leica RM 2145), stained with Azan according to Geidies, and mounted in Roti-Histokitt (Carl Roth). Sections were digitized with a Nikon Eclipse 90i microscope equipped with a digital Nikon DS2-MBW camera. In addition, selected preparations were analyzed with a Leica SP5 II confocal laser scanning microscope. Autofluorescence microscopy was used to visualize the morphology of the first and second antenna as suggested by Haug et al. (2011). Digital images were processed with Adobe Photoshop, if necessary. Only global picture enhancement features (i.e., brightness and contrast) have been used. 3D reconstructions base on paraffin thick-section series. Alignment and reconstruction were performed with AMIRA 5.2 (Visage Imaging). In each section, contours of the neuropils were traced, out of which a 3D model was generated. For compiling the diagrams, we used Adobe Illustrator CS4. The neuroanatomical nomenclature of this manuscript is based on Sandeman et al. (1992), Richter et al. (2010), and Loesel et al. (2013) for the description of the neuropils, cell cluster, and tracts. Moreover we propose new terms to facilitate homologization of certain characters between malacostracan crustaceans and hexapods.

RESULTS

The position, general appearance and a schematic of the syncerebrum of *S. entomon* is shown in **Figure 2**. The neuraxis is prominently bent dorsally in the region of the esophageal connectives (**Figures 2B,C**, dotted line in **3A**), resulting in an L-shape in which the brain lies approximately perpendicular to the ventral nerve cord. All following descriptions refer to the body axis. Three neuromeres can be identified from dorsal to ventral. The brain is dominated by the dorsal most protocerebrum (PC), in particular by the protrusion of the lateral protocerebrum (lPC) and optN (**Figures 2B,C**, **3**, **4A**).

OPTIC NEUROPILS

Visual afferents from the retina (R; **Figures 3A**, **4A**) project *via* long optic nerves (oNv, i.e., up to 13 mm in larger specimen) laterally into the brain and supply four consecutive and closely associated optN (**Figures 2**, **3**). These are surrounded by a cell cortex comprising the cell cluster 1–3 [according to the terminology by Sandeman et al. (1992)]. The first, most distal neuropil, the lamina (La) is displaced distally away from the brain and interconnected with the second neuropil (medulla, Me) by the (outer) optic chiasm (oCh, **Figures 3B,B′**). The connectivity of the latter and the third neuropil (lobula, Lo) could not be clarified (iCh?; **Figures 3B,B′′**) due its close proximity to the lPC. The lamina is weakly immunoreactive for the antibodies used. In contrast, the medulla is intensively labeled with antisera against FMRF-amides (RF), serotonin (5HT), and synapsin (SYN), yet is unstructured. The lobula is accompanied by a small fourth neuropil, the lobula plate (LoP), which only could be vizualized unequivocally by anti-synapsin labeling (LoP; **Figure 3B′′**). Its connectivity with medulla, lobula, and lPC could not be clarified with certainty.

LATERAL PROTOCEREBRUM

The lobula and LoP are closely associated with a neuropil complex comprising the lPC (**Figures 2C**, **3C,D**). Within the lPC, no clearly demarcated neuropil regions are distinguishable. However, anti-synapsin labeling reveals an intensively stained region surmounting the lPC dorsomedially like a cap, the hemiellipsoid body (HE; **Figure 3C**). This neuropil is formed by neurites from neurons whose somata are located in three bulb-like and very distinct spherical clusters located dorsolaterally, dorsally and dorsomedially of the protocerebral complex (cluster 5′–5′′′, **Figure 3C′**). Another neuropil of the lPC, the medulla terminalis (MT), has a homogeneous texture and is innervated by a large but indistinct posterolateral cluster (cluster 4; **Figures 3C,C′**). Both neuropils of the lPC are supplied by a prominent neurite bundle emerging from the deutocerebrum (DC), the projection neuron tract [PNT according to Loesel et al. (2013); olfactory globular tract according to the traditional terminology of Sandeman et al. (1992); **Figure 2C**, dotted circles in **3C**]. This tract gives rise to a small branch innervating the hemiellipsoid bodies (asterisk in **Figure 3C**), the remainder could not be traced any further but most likely ends within the medulla terminalis (double asterisk in **Figure 3C**). A second tract (protocerebral tract, PT, solid circles; **Figure 3C**) connecting the lPC with the “central” brain is located laterally to the medulla terminalis. The protocerebral tract is composed of two branches, one of which terminates within the lPC whereas the other proceeds toward the optN.

MEDIAN PROTOCEREBRUM

The median protocerebrum (mPC) is easily identified in sections by the central complex that is composed of three distinct neuropils: the central body (CB), the lateral accessory lobes

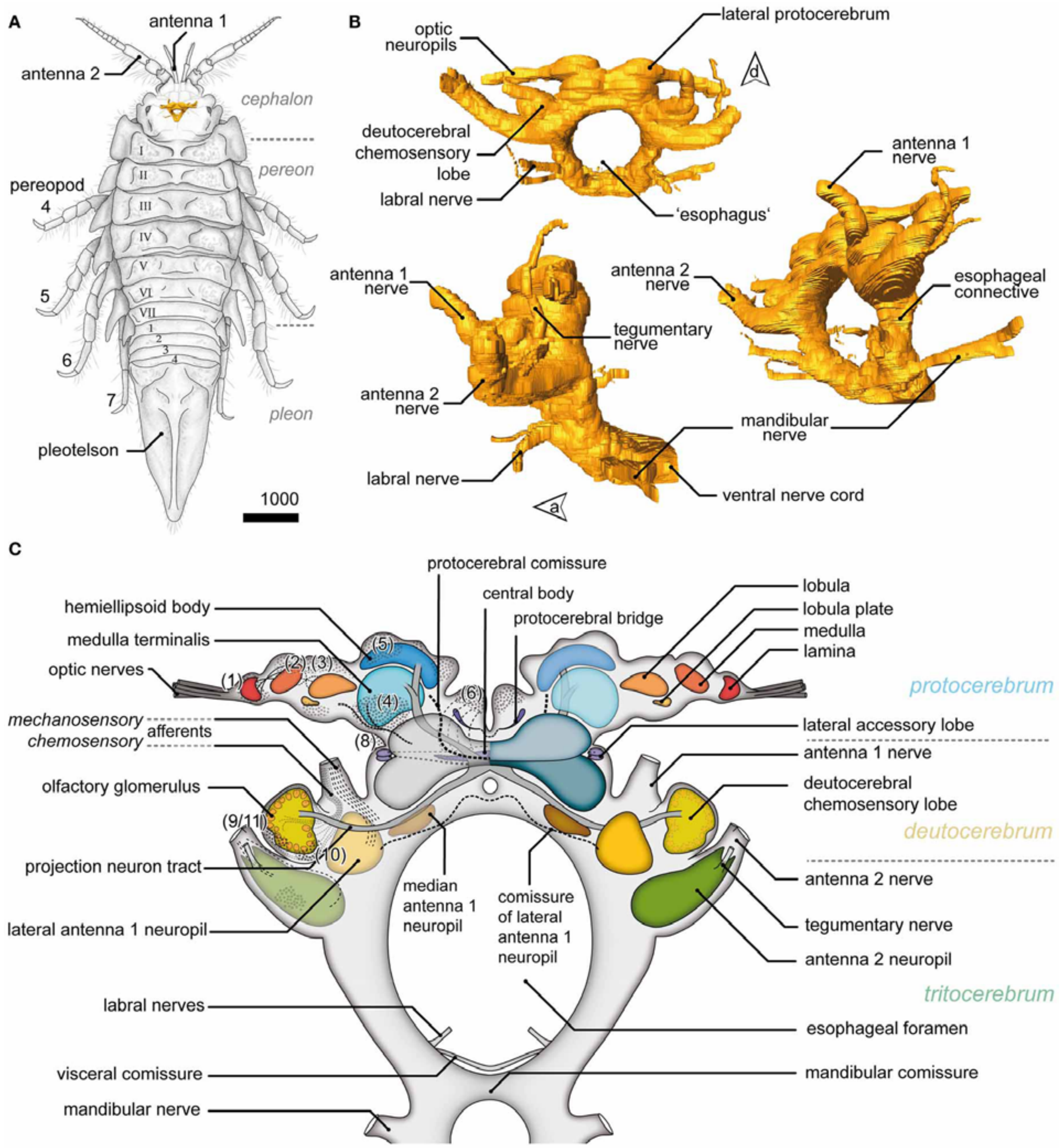

FIGURE 2 | General overview of the brain in *Saduria entomon*. (A) A sketch of *Saduria entmon* showing the position and relative proportion of the brain. **(B)** 3D reconstruction of the brain from frontal (top left), lateral (bottom left) an ventrofrontal (right) showing the major neuromeres and sensory afferents. **(C)** Semidiagrammatic representation of *S. entomons*' brain.

(lal) and the protocerebral bridge (PB) (**Figures 2C**, **4**). Two cell clusters are associated with this complex. The unpaired CB extends transversely across the midline and provides a conspicuous cigar-shaped landmark in the median brain, just dorsal to the cerebral artery (CA) which pierces the brain in an anterior—posterior direction. It is intensively labeled by all antibodies used here but does not show any obvious subdivisions. The neuropil is completely embedded between several commissural neurite bundles (solid circles in **Figures 4C,D,F**). Further, it is surrounded by a number of somata which are likely glia

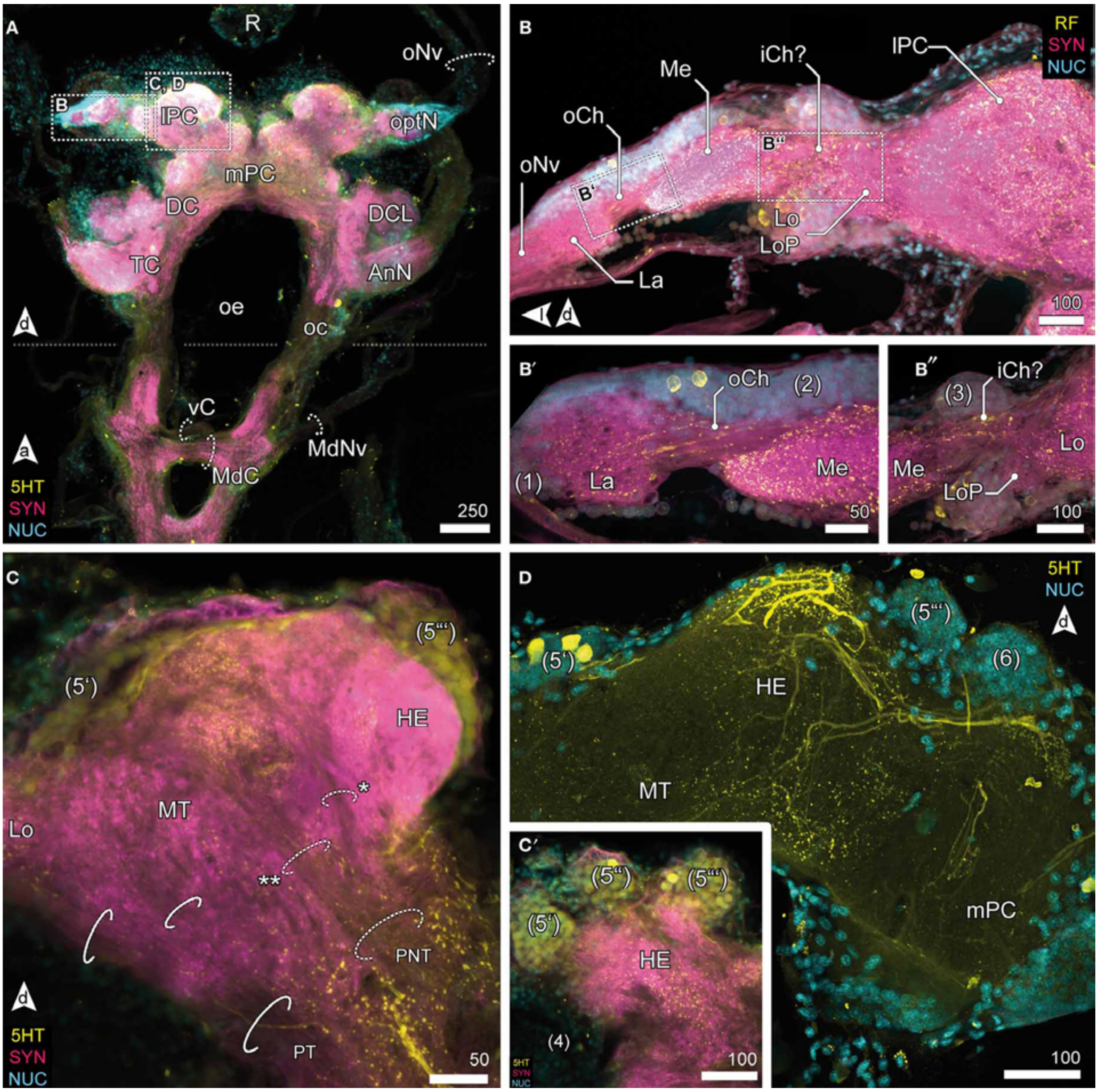

FIGURE 3 | Optic neuropils and the lateral protocerebrum. (A) Overview of the brain from posterior and dorsal view of the anterior part of the VNC. The dotted line indicates the change of orientation between brain and nerve cord. The optN are supplied by the R by oNv. Two commissures connect the oc; an anterior vC, and the posterior MdC out of which the MdNv emerge. **(B)** Posterior view of the optN **(B′)** Magnification of connection between La and Me, the oCh. **(B″)** Magnification of connection between Me and Lo. The connectivity remains unresolved, thus the presumptive iCh is labeled with "?." The Lo is accompanied by a small loP neuropil. **(C)** The IPC in different section planes, center **(C)** and anterior end **(C′)**. The IPC is innervated by the PNT, giving off a branch innervating the HE (asterisk), the remainder proceeds into the MT (double asterisk). The PT connects optN and MT with the mPC. **(C′)** The HE is innervated by neurons located in three bulb-like clusters (5) anterodorsal to the neuropil. **(D)** The HE is heavily innervated by 5HTir neurons located in cluster (5′), giving of fine branches into the deeper layers and MT.

cells (**Figures 4B–D,F**), as none of them exhibited immunoreactivity for the antibodies used. The neuropil is supplied by neurites emanating from a paired anterodorsal cell cluster [(6) in **Figures 4B–E**]. These neurites form synapses in a small paired neuropil, the PB (**Figures 4B,D,F**) and project in four distinct tracts (W, X, Y, Z, **Figure 4E′**; dotted circle in **D**) to the CB. Both PB neuropils are connected by a commissure that shows no immunoreactivity for FMRF-amides but contains at least one large serotonergic neurite (dotted circles in **Figures 4B,F**). The lateral accessory lobe, a bilaterally paired neuropil is located

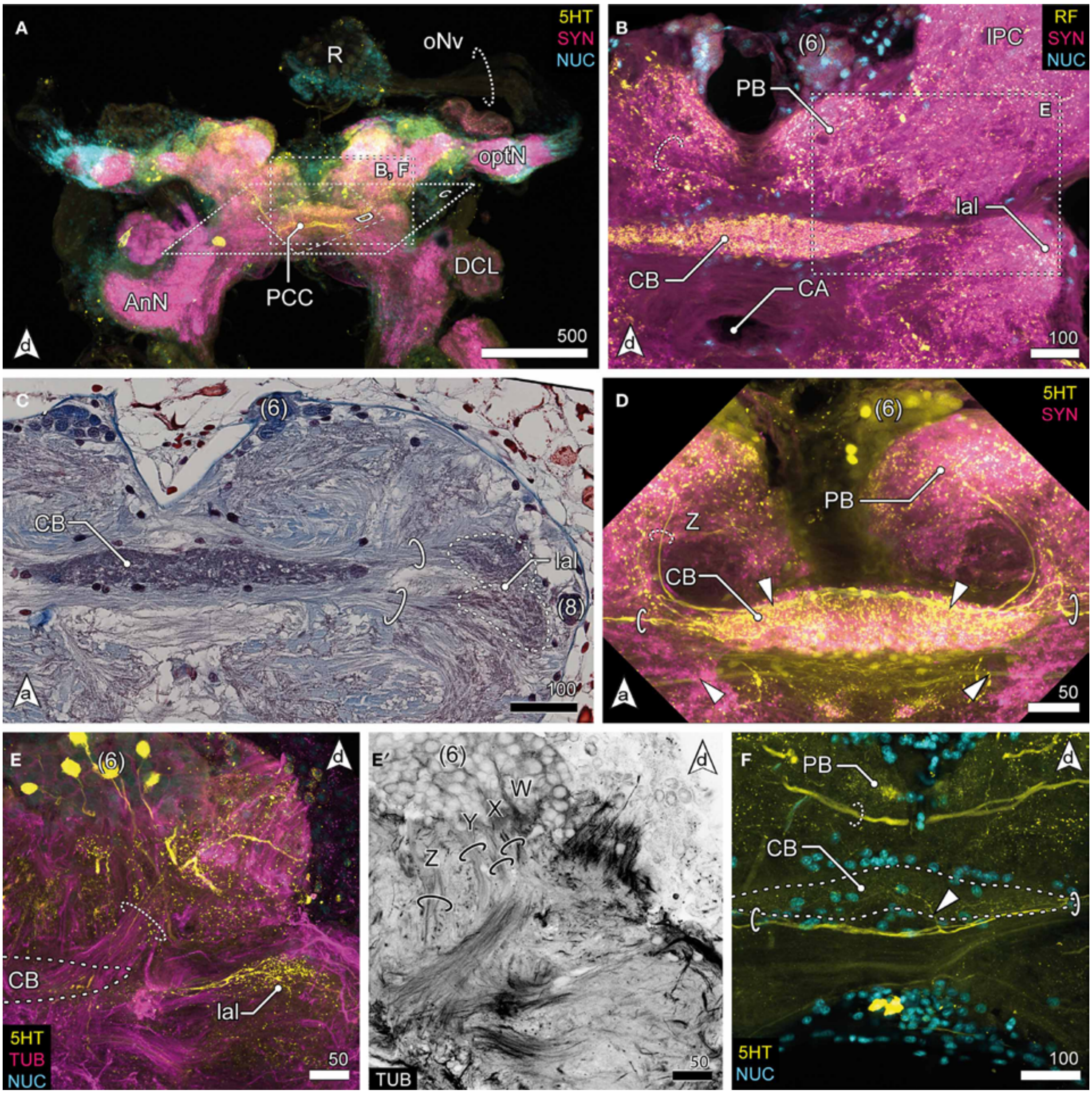

FIGURE 4 | Median protocerebrum and central complex. (A) Overview of the brain from posterior. A large 5HTir PCC connects both hemispheric IPC ventrally to the CB. **(B)** The central complex labeled against RF-amides showing the anterodorsal cluster 6, the PB, CB and lal, frontal section. The CB is ensheathed by a couple of somata, none of which showing RFir (compare with **F**). The PB is interconnected by a commissure (dotted circle). **(C)** Ventral extensions of (6) and (8), CB and lal. A couple of somata are distributed around the CB. The bipartite lal are interconnected by two large commissures (solid circles), horizontal paraffin section. **(D)** Frontal section of CB. 5HTir of commissures interconnecting the lal (solid circles). The CB is also innervated by neurons (dotted circle, Z) from (6), corresponding to tract Z [according to Utting et al. (2000); dotted circle, see **E′**] **(E)** Anterolateral region of the mPC showing the anterior unit of the lal, also a small part of the PNT is visible (dotted circle), frontal section. The CB (posterior to this section plane) is indicated by a dotted line. **(E′)** Single channel image showing TUBir of projections of cluster 6 corresponding to tract W, X, Y and Z, innervating the CB and lal. **(F)** Frontal section of CB. 5HTir of PB commissure (dotted circle) and arborizations of neurons from (8) (arrowhead) bypassing the CB ventrally (solid circles).

laterally to the CB and is innervated by neurons from cluster (6) and from a small bulb-like cluster (8) protruding laterally from the brain (**Figure 4C**). It is distinctly immunoreactive for 5HT but its bipartite nature is more clearly seen in histological sections, (dotted circles in **Figure 4C**). Two large commissures, anterior and posterior to the CB, interconnect the accessory lobes (solid circles in **Figures 4C,D,F**) and extend arborizations into the midline neuropil (arrowhead in **Figure 4F**). Anti-serotonin-labeling reveals another large commissure connecting both hemispheres of the lPC (PCC, **Figure 4A**).

DEUTOCEREBRUM

The DC, though small in proportions, is characterized by distinctive lateral outswellings comprising the deutocerebral chemosensory lobes (DCL; olfactory neuropils or olfactory lobes according to the traditional terminology; **Figures 2**, **5**). This nearly spherical neuropil of approximately 250 μm in diameter protrudes laterally from the brain and is thus easily detached during the preparation procedure. From section series, we estimate that it consists of about 80 small, distinct neuropil subunits of roughly spherical shape, the olfactory glomeruli (og, **Figures 2C**, **5C–G**). The glomeruli are arranged radially around the periphery of the DCL and surround a coarse neuropil (cN) of loose neuronal processes. They are further divided into two distinct domains, a distal cap and proximal base. The cap is strongly labeled by the antisera against neuropeptides whereas in the base, SYN immunoreactivity predominates (**Figures 5E,F**). The DCL is supplied by afferents from aesthetascs situated on the distal segment of the antenna 1 (**Figure 5B**) *via* the antenna 1 nerve (A1Nv, **Figures 2C**, **5C,D**). Afferents (cA) enter the chemosensory lobes' og from the periphery (arrowheads in **Figure 5D**) and apparently also from within the lobe (double arrowheads in **Figure 5D**) through a median foramen (mF). The latter is a quite remarkable finding that requires further investigation. A small cell cluster (9/11) is located ventrolaterally and houses local interneurons whose neurites enter the neuropil between two glomeruli through a lateral foramen (lF, **Figure 5D**). A posterioventral extension of this cluster also innervates medial parts of the DC (arrowhead in **Figure 5C**). 5HT labeling reveals a single prominent serotonergic neurite innervating several og, yet, the corresponding soma has not been found (**Figure 5G**). Output from the DCL is provided by a large tract of projection neurons (PNT) emerging from the center of the neuropil through the mF (**Figures 2C**, **5C,D**). The corresponding somata are located in a small irregularly shaped cluster (10) posteroventrally to the DCL (**Figure 5C**). The PNT proceeds further into the mPC to target neuropils of the lPC (**Figures 2C**, **3C**). At the level of and slightly posterior to the CB, both hemispheric branches of the PNT approach the midline and form a chiasm in which several neurites enter the contralateral side (Stemme and Eickhoff, pers. commun.). Apart from the presumptive chemosensory afferents entering the DCL, the antenna 1 nerve gives rise to another branch (mA) dorsomedially that proceeds further into the DC innervating the lateral antenna 1 neuropil (LAN), an undivided paired neuropil of inconspicuous shape located medially to the DCL (**Figures 2C**, **5A,C,D**). These neuropils are interconnected by a thin commissure (not shown). Between the lateral antenna 1 neuropil and CB, a diffuse bilaterally paired neuropil region is visible showing an irregular immunoreactive patterning. Its location suggests that it may constitute the median antenna 1 neuropil (MAN), although it is not clear from our specimens whether this region actually receives any input from antenna 1 (**Figures 2C**, **5A**).

TRITOCEREBRUM

Aside the protocerebral neuropils, the brain of *S. entomon* is dominated by the tritocerebral neuropils protruding anterolaterally from the esophageal connectives and targeted by a large nerve that is supplied by receptors on antenna 2, including the apical cone (**Figures 2C**, **6A**). The nerve is mainly associated with the antenna directly but a smaller lateral branch can be traced to muscles at the base of antenna 2 (arrowheads in **Figure 6D**). Close to the esophageal foramen the nerve thickens and enlarges to the distinct spindle-shaped antenna 2 neuropil (AnN, **Figures 2B,C**, **3A**, **6B–D**). This neuropil is labeled by all antibodies used. In particular anti-synapsin labeling reveals a rather complex structure reminiscent of a microglomerular organization (inset, **Figure 6C**). In addition, in parts the neuropil displays a transverse striation (dotted circles in **Figure 6B**) although the current data is not conclusive. The area where parts of the A2Nv enter the neuropil, i.e., the tip, appears to be grooved or sunken in (double arrowhead in **Figures 6B,D**). A small branch of the antenna 2 nerve bypasses the antenna 2 neuropil dorsomedially (arrowheads in **Figures 6C,D′**). It emerges from the medial region of the antenna 2 neuropil, close to the esophageal connectives. A thin nerve (tegumental nerve, TNv; **Figures 2B,C**, **6C,D′**) emerges from the antenna 2 neuropil posteriorly and ascends dorsally, bypassing the optN. The esophageal connectives are interconnected by two commissures ventrally to the tritocerebral domain that merges with the ventral nerve cord. The anterior visceral commissure (vC) is thin, gives rise to the labral nerves (LNv) anteroventrally and is located directly anteriorly to the larger mandibular commissure (MdC, **Figures 3A**, **6E**).

DISCUSSION

The general morphology of the brain in *S. entomon* presented above broadly equals what has been reported previously for other representatives of the Isopoda. However, the most pervasive difference is found in the peripheral and central olfactory pathway when compared with terrestrial representatives.

ANTENNA 1

Malacostracan Crustacea are typically equipped with two pairs of antennae, a first pair (antennule or antenna 1) associated with the DC, and a second pair (antenna or antenna 2) associated with the tritocerebrum. In addition to bimodal chemo- and mechanosensilla distributed along the length of the antenna 1, the distal segment of the first antennae bears an array of specialized chemoreceptive sensilla housing the branched dendrites of olfactory sensory neurons, the aesthetascs (Hallberg et al., 1992, 1997; Hallberg and Hansson, 1999; Hallberg and Skog, 2011; Schmidt and Mellon, 2011). As shown in previous studies (Kovalevskij, 1864; Pynnönen, 1985), *S. entomon* possesses well developed antennulae, with their distal segment equipped with aesthetascs. Although sparsely innervated by dendritic processes, their general morphology strongly resembles those of other marine crustaceans [e.g., *Panulirus argus*: (Laverack, 1964); *Homarus americanus*: (Shelton and Laverack, 1970); *Idotea baltica*: (Guse, 1983); reviewed in Hallberg and Skog (2011)], suggestive of a chemoreceptive function. They are located in a row of up to 30 tufts on the ventral edge of antenna 1 pointing anteriorly, with each tuft bearing a pair of aesthetascs that is accompanied by three accessory setae (Pynnönen, 1985). Thus, with approximately 60 aesthetascs, *S. entomon* has a relatively low number compared to other marine malacostracans but is still undercut, e.g., by the brachyuran *Uca*

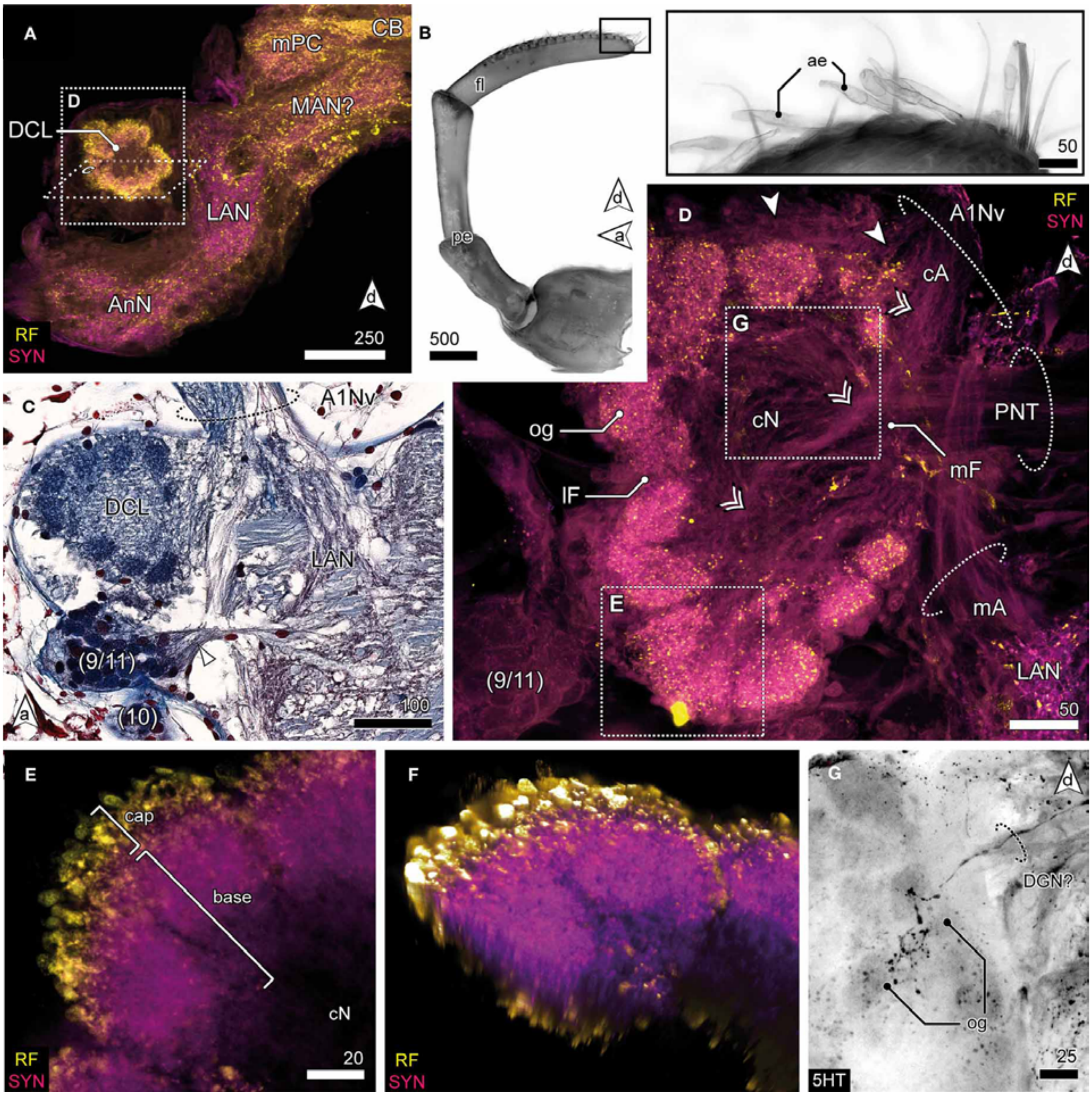

FIGURE 5 | Deutocerebrum and the deutocerebral chemosensory lobe. **(A)** DC and TC as shown in anti-RF and anti-SYN labeling. The DCL protrudes laterally from the DC, flanking the LAN. **(B)** The distal antennal segment bearing several tufts composed of 3 accessory setae and 2 aesthetascs (inset) giving rise to A1Nv; UV-autofluorescence. **(C)** Horizontal paraffin section of the DC showing innervation of DCL by A1Nv (dotted circle), cluster (9/11) and (10), and the LAN. **(D)** The DCL as reveal in anti-AST and anti-SYN labeling showing its glomerular organization surrounding the cN. A1Nv sends off two branches into the DC, presumptive chemosensory afferents (cA) innervate the og from the periphery (arrowhead) but also from the center (double arrowhead). Another branch proceeds toward the LAN and carries mechanosensory afferents (mA) and probably also efferents. A cluster of local interneurons (9/11) is located posteriolateral to the DCL and sends out neurites into the DCL through a lateral foramen (lF; not visible in this section plane). Output is provided by projection neurons (PNT) through a medial foramen (mF). **(E)** Close up of a single glomerulus illustrating the subdivision into a cap region showing intensive neuropeptide-ir and a base with a predominant anti-SYN labeling. **(F)** Volume rendering of image-stack shown in **(E)**, showing that the center of the glomerulus is devoid of neuropeptides-ir while the periphery is intensively stained. **(G)** 5HTir of the DCL's surface showing a single prominent serotonergic neurite with multiglomerular innervations.

pugnax (Brachyura, Decapoda) with only about 26 aesthetascs (Beltz et al., 2003). At the other end of the range *Panulirus interuptus* (Achelata, Decapoda) was determined to house up to 1786 aesthetascs per antenna 1 (Beltz et al., 2003). When dealing with such numbers, it has to be noted that during the course of a crustaceans' life aesthetascs are continuously added while the animal grows (Beltz et al., 2003). In contrast, oniscid Isopods underwent some radical modifications of this general pattern

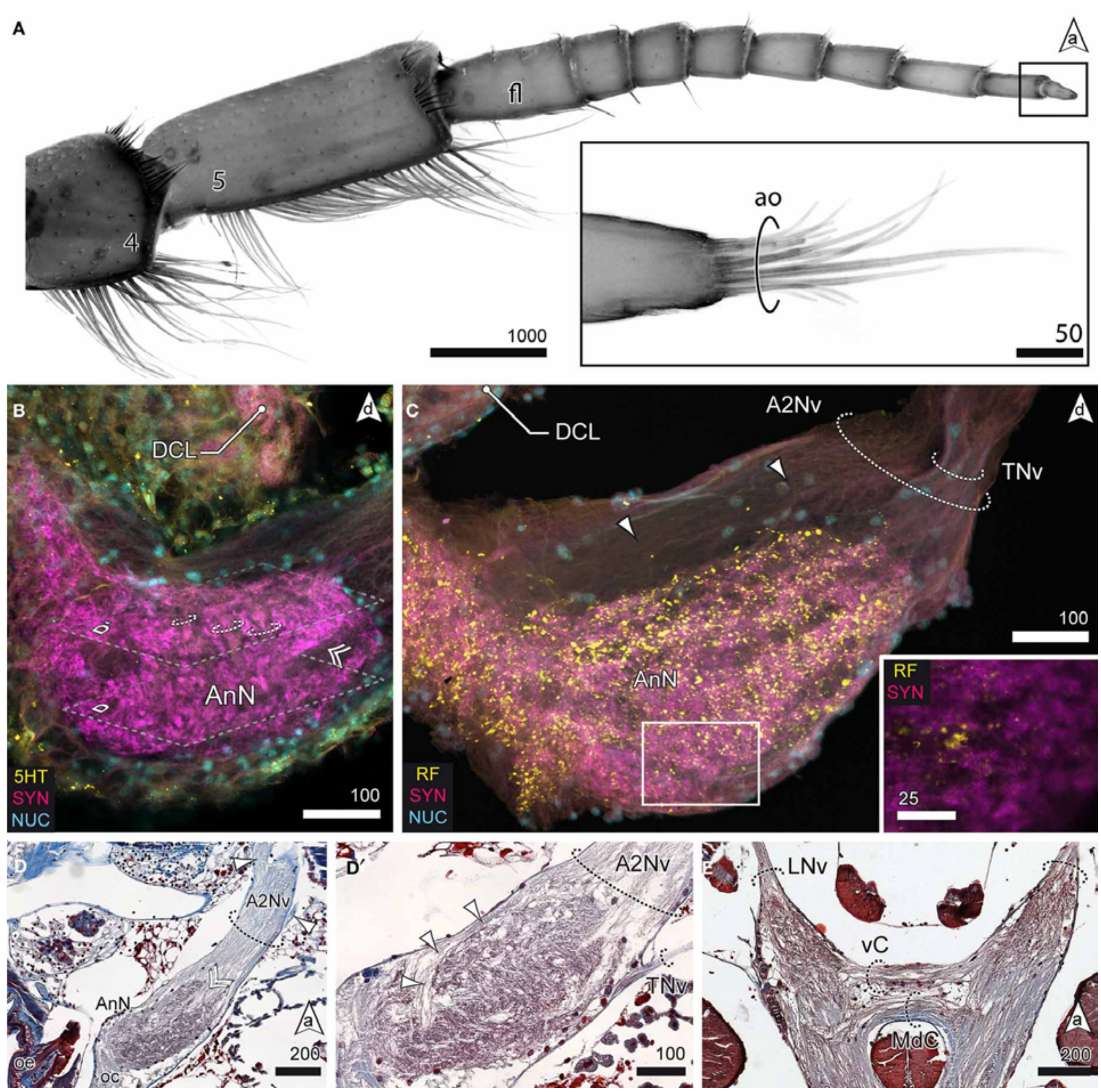

FIGURE 6 | The tritocerebrum. (A) UV-autofluorescence of antenna 2 and the tip showing the last two antennal segments and fl bearing the ao (inset). **(B)** AnN as revealed in anti-5HT and anti-SYN labeling. Parts of A2Nv enter the neuropil in a groove like depression (double arrowhead, compare with **D**). The AnN shows traces of repetitively arranged neurite bundles crossing the neuropil in a perpendicular manner (dotted circles). **(C,D′)** The TNv projects into the AnN posteriolaterally while parts of the A2Nv bypass the neuropil anterodorsally and the target domains in the posterior parts of the neuropil. SYNir illustrates the microglomerular organization of the AnN (inset **C**). **(D)** Nerves emerging from A2Nv target muscles at the base of antenna 2 (arrowheads). Parts of A2Nv enter the neuropil in a groove like depression (double arrowhead). **(E)** Anterior part of the VNC showing two commissures connecting the oc. An anterior vC giving rise to LNv and the posterior MdC.

[reviewed in Schmalfuss (1998)]. For example the xerophilic desert isopod *Hemilepistus reaumuri* is characterized by a considerable diminution of the first antennae leaving only a very small appendage that is, yet, not without function. Haug and Altner (1984) have shown that in the woodlouse *Porcellio scaber*, the tips of the first antennae are equipped with about 15–20 peg sensilla, which the authors interpret as hygroreceptors, and so called "olfactory hairs" (i.e., aesthetascs) have been reported for *Oniscus asellus* (Ábrahám and Wolsky, 1930). In a behavioral study Zimmer et al. (1996) have demonstrated that *P. scaber* apparently is able to perceive odors and orients toward a food source. However, it is not evident if the animal actually uses antennular olfaction as Hoese and Schneider (1990) stress the importance of constant ground contact of the second antennae while the animal is moving. Nonetheless, a chemoreceptive function of the first antennae of terrestrial isopods cannot be ruled out.

DEUTOCEREBRAL CHEMOSENSORY LOBES

The evolutionary size reduction of the first pair of antennae in terrestrial isopods has decreased the sensory input to the DC which resulted in greatly dimished deutocerebral brain areas, and left in most cases not a single trace of neither chemosensory nor mechanosensory areas. Gräber (1933) analyzed the brains of *O. asellus*, *P. scaber*, and *Armadillidium cinereum*, and was not able to identify particular deutocerebral neuropils. Walker (1935) describes a small, untextured neuropil in the ventrolateral DC of *O. asellus* that he considers an olfactory lobe, although such function has not yet been confirmed in behavioral essays. A recent study on the brains of *P. scaber*, *A. vulgare*, and *H. reaumuri* again corroborates the general scheme that the DC in terrestrial isopods is considerably minimized in comparison to aquatic isopods (Harzsch et al., 2011).

In *S. entomon*, at least two neuropils that are associated with the first antenna are present and are comparable to what is found in other malacostracans. The ovoid DCL (i.e., the olfactory lobe) consists of a peripheral array of about 80 radially arranged synaptic spherical fields surrounding a cN of neuronal processes comprising the neurites of local interneurons and projection neurons. These og are subdivided into two distinct layers, a distal cap and a proximal base. For other malacostracans it has been shown that this division mirrors a functional segregation as it is the result of a regionalized innervation pattern of local inter- and projection neurons (Schmidt and Ache, 1997; Schachtner et al., 2005; Polanska et al., 2012). The glomerular organization of DCL as exemplified in *S. entomon* is found in many other malacostracans although variations of this scheme exist. It also has already been regarded to be part of the malacostracan ground pattern (Kenning et al., 2013). In the Decapoda for example, the neuropil experienced several modifications with respect to the general morphology and neuronal architecture of its compartments, in terrestrialized species in particular. In the giant robber crab *Birgus latro*, a terrestrial anomuran, the neuropil is a multilobed complex of more than 1000 markedly elongated, cylindrical og, constituting for roughly half of the total brain volume (Krieger et al., 2010). Moreover, in reptant decapods the glomeruli gain an additional third layer, the subcap, that is located between the cap and base and receives inputs from local olfactory interneurons (see, e.g., Schachtner et al., 2005; Harzsch and Hansson, 2008; Krieger et al., 2010, 2012). This subcap is connected with a neuropil that is only known from reptant decapods, the deutocerebral accessory lobes (Polanska et al., 2012; Sandeman et al., in press). This intricate organization of several deutocerebral neuropils indicates rather sophisticated olfactory systems. As pointed out, our knowledge on the olfactory pathway in Crustacea is mainly founded on studies on Decapoda. Thus, only little information is available for other representatives of the Malacostraca, specifically the Peracarida. Concerning isopods, Harzsch et al. (2011) analyzed various terrestrial representatives but also included a single marine species in the study, the closely related valviferid *I. baltica*. Like *S. entomon*, *I. baltica* possesses aesthetasc bearing first antennae that are associated with a DC housing glomerular DCL. Gräber (1933) examined two species of *Gammarus* (Amphipoda), in both of which, *G. pulex* and *G. fluviatilis* he found rather large DCL housing glomeruli. Within Malacostraca, a similar organization is found in the brains of Stomatopoda (Derby et al., 2003), Euphausiacea (Johansson and Hallberg, 1992), and even Phyllocarida (Kenning et al., 2013), all of which possess DCL that are build up of spherical og, though much fewer in numbers than in Decapoda.

PROJECTION NEURON TRACT

The PNT, relaying information from the DCL to higher-order processing areas in the lPC (Sandeman et al., 1992; Sullivan and Beltz, 2004), has sparked many discussions as it potentially is of phylogenetic significance concerning the relationships of insects and crustaceans (Strausfeld, 2012). A decussation of this tract in Isopoda has long been negated, although this feature is regarded as a character that was already present in the ground pattern of the Malacostraca (Kenning et al., 2013). According to Hanström (1968), the PNT in the litoral species *Ligia occidentalis* interconnects only ipsilateral neuropils. Equally, in true terrestrial isopods (e.g., *O. asellus* and *P. scaber)* a chiasm is reported to be missing (Gräber, 1933; Walker, 1935), whereas in the amphipod *G. pulex* the PNT features a contralateral connection (Gräber, 1933). As this tract also shows a prominent chiasm in *S. entomon*, it is hard to draw a plausible conclusion with only limited possibilities of comparison, not to mention the unresolved phylogeny of the Isopoda. Keeping in mind that a decussation provides an entirely new level of complexity in neuronal integration pathways, it is hardly conceivable that it evolved convergently in different peracarid representatives. Therefore, we believe that the most likely fate of the chiasm was a reduction of contralateral fibers in the oniscid line, probably due to the aforementioned reduction of antennular input that had a cascading effect on associated structures.

LATERAL PROTOCEREBRUM

Like the DCL, the neuropils of the lPC have received some attention by researchers and have been described in a number of crustacean taxa. The lPC is composed of the medulla terminalis and the HE and especially the latter has been thoroughly investigated as it seem to play a key role in olfactory learning and constitutes the site of olfactory and multimodal integration (Maynard and Dingle, 1963; Maynard, 1965; Maynard and Yager, 1968; Sullivan and Beltz, 2004; Strausfeld, 2012; Loesel et al., 2013). Information on the organization of the lPC in Isopoda or even Peracarida is scarce but some of the figures provided by Harzsch et al. (2011) allow to draw conclusions on the morphology of the neuropils in question. In all three terrestrial taxa investigated, a neuropil reminiscent of a HE seems to be present [see Figures 5, 6 in Harzsch et al. (2011)], contradicting descriptions of Hanström (1968). However, architecture and innervation patterns are unknown. Corresponding neuropils have also been documented in *Mysis relicta* and *Callomysis maculata* (Mysidacea) but instructive descriptions are missing Hanström (1968). Whereas in *S. entomon* the neuropil shows no signs of subdivision, studies on *Coenobita clypeatus* (Anomura) revealed that their HE is intricately structured and features several subdivisions into multiple cap-neuropils mounting a core neuropil. In these, ascending information from both DCL is relayed to a dense, rectilinearly multilayered network (Brown and Wolff, 2012; Polanska et al., 2012; Wolff et al., 2012), and may thus be involved in higher-order olfactory processing.

LATERAL ANTENNA 1 NEUROPILS

In Malacostraca, mechanosensory input from the antenna 1 is relayed to the lateral antenna 1 neuropil. At least for decapods, it has been shown that it also receives afferents from the statocyst and non-aesthetasc chemoreceptors (Sandeman and Denburg, 1976; Yoshino et al., 1983; Roye, 1986; Blaustein et al., 1988; Schmidt and Ache, 1993), and is involved in controlling the movements of the first antenna (Maynard, 1965; Roye and Bashor, 1991; Schmidt and Ache, 1993, 1996). For several representatives of Malacostraca it has been noted that this neuropil shows a horseshoe-like appearance, and Kenning et al. (2013) considered this organization as plesiomorphic for this taxon. However, this trait seems to have been lost in the isopod clade. Although a lateral antenna 1 neuropil is present and likely takes part in controlling the movements of the first antenna, a contribution to the equilibrium sense is unlikely as statocysts in Isopods are located in an anterolateral process of the cephalon at the level of and just anterior to the compound eyes and being associated with a not further specified region in the lPC (Wenig, 1903; Walker, 1935).

TRITOCEREBRAL NEUROPILS

Like in other malacostracans, the second antenna of *S. entomon* relays its input into a comparatively large neuropil area, the antenna 2 neuropil. Yet, here it is of quite conspicuous texture showing a microglomerular organization like it has been reported for *H. reaumuri* (Harzsch et al., 2011) and other terrestrial representatives of the Isopoda (Gräber, 1933; Walker, 1935), an aspect that may been overlooked in the study of Harzsch et al. in *A. vulgare* and *P. scaber*. While a glomerular neuropil organization is often associated with a chemoreceptive function (Strausfeld, 2012), it is known from various other malacostracan crustaceans that their antenna 2 neuropils are longitudinally subdivided into repeated units, and receive the somatotopic representation of the chemo- and mechanosensory sensilla on antenna 2 (Tautz and Müller-Tautz, 1983; Zeil et al., 1985; Sandeman and Varju, 1988; Krieger et al., 2012). Interestingly, this is also the case in the marine *I. baltica* (Harzsch et al., 2011). The finding of a microglomerular organization of the antenna 2 neuropil in the closely related *S. entomon* but not an (obvious) longitudinal subdivision raises questions about its sensory significance. As the first antennae are greatly reduced in oniscid isopods, the second antennae are thought to function as the major sensory organs [reviewed in Schmalfuss (1998)] with their unstalked eyes playing a subsidiary role. Similar to their marine relatives, all segments of their second antennal pair carry numerous mechanoreceptive sensilla. In addition, the terminal segment bears a tuft of robust sensilla, the apical organ or apical cone which is interpreted as detecting both mechanical and chemical stimuli (Mead et al., 1976; Alexander, 1977; Hoese, 1989; Hoese and Schneider, 1990; Schmalfuss, 1998). In the desert isopod *H. reaumuri*, the apical organ has even been suggested to respond to both olfactory and gustatory stimuli (Seelinger, 1977, 1983). Although responses to distant olfactory stimuli have not yet been tested behaviorally, their apical organs likely play a key role in the perception of chemical signals involved in social recognition, family cohesion, congregation, and communication (Fischbach, 1954; Kuenen and Nooteboom, 1963; Linsenmair, 2007). To what extend this might also hold true for a marine representative remains uncertain.

NEUROETHOLOGICAL CONSIDERATIONS AND CONCLUSION

Our findings suggest that *S. entomon* not only has the morphological substrate to perceive but also to process olfactory stimuli. However, without lab-based ethological investigations, statements on the animals' sensory capabilities are pure conjecture. Yet, it may nevertheless be enlightening to discuss previous electrophysiological investigations of the eyes, ultrastructural examinations of the aesthetascs, and our own findings with regard to the animal's life style. *S. entomon* is an opportunistic and omnivorous nocturnal scavenger that lives on dead fish and other carcasses as it may find on the sea bottom but also actively preys on amphipods, conspecifics, and other isopods (Green, 1957; Haahtela, 1990; Leonardson, 1991). Whereas younger populations of *S. entomon* migrate into shallow water coastal areas during the winter, the main depth of occurrence is 50–85 m and even depths down to 290 m have been reported. The eyes show a relatively high sensitivity with maximum absorption close to the wavelength of maximum light transmittance in their habitat waters (Lindström et al., 1991). However, the dorsal position of the eyes in *S. entomon* and low light levels in its habitat raises the question if this animal's feeding behavior is mostly visually guided or rather if other senses are substantial for the survival of this species. Preliminary experiments by Pynnönen (1985) suggest that *S. entomon* is chemically attracted to food sources and that the aesthetasc equipment of antenna 1 is necessary for a directed movement toward a food source. Observations in the field as well as in the laboratory indicate that *S. entomon* does react and orients toward dead fish and fluid homogenates of conspecifics but that tracking movement, however, is fairly undirected and random (personal observations by the authors). This requires further investigations in bioassays to evaluate which role chemical stimuli play for the feeding behavior. A corresponding experiment has been conducted using *Scopelocheirus onagawae* (Amphipoda) that demonstrated nicely how the animals react to and congregate at agar blocks saturated with a certain synthetic amino acid mixture, imitating rotting fish (Ide et al., 2006).

The evidence discussed above demonstrates that in Isopoda a significant reconfiguration of appendages and brain areas involved in olfaction has taken place during the evolutionary conquest of land. Along these lines, it may be well worth exploring which other adaptations the Isopoda might have evolved in their visual, chemo- and mechanosensory systems when considering the dramatically different sensory ecology, e.g., in a challenging habitat like the abyssal plains or in animals with more derived life styles such as parasitic isopods.

AUTHOR CONTRIBUTIONS

Matthes Kenning designed and performed the experiments, and analyzed the data under supervision of Steffen Harzsch. Both authors wrote the manuscript, discussed the results and implications and commented on the manuscript at all stages.

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SHORT COMMUNICATION

Heading which way? Y-maze chemical assays: not all crustaceans are alike

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Abstract In a world full of chemicals, many crustaceans rely on elaborate olfactory systems to guide behaviors related to finding food or to assess the presence of conspecifics and predators. We analyzed the responses of the isopod *Saduria entomon* to a range of stimuli by which the animal is likely to encounter in its natural habitat using a Y-maze bioassay. In order to document the efficiency of the experimental design, the same bioassay was used to test the behavior of the crayfish *Procambarus fallax* whose ability to track odors is well documented. The crayfish performed well in the Y-maze and were able to locate the source of a food-related odor with high fidelity. The isopod *S. entomon* reacted indifferently or with aversion to most of the stimuli applied. In 1800 trials, only four out of 15 different stimuli yielded statistically significant results, and only one odorant was found to be significantly attractive. The findings raise several questions whether the stimuli presented and/or the experimental setup used represents an ecologically relevant situation for *S. entomon*. In each instance, our experiments illustrate that established methods cannot be readily transferred from one species to another.

Keywords Isopoda · *Saduria entomon* · Decapoda · Crayfish · Olfaction · Behavior · Flow channel

Introduction

The ability to obtain information about the environment is the fundamental basis for an organism's ecological interactions. In aquatic organisms, chemical stimuli provide highly important cues for survival and reproduction because they indicate the availability of resources such as food, shelters or substrates for larval settlement, and allow assessing the presence of predators, conspecifics and potential mates (Derby and Sorensen 2008; Thiel 2011; Wyatt 2011; Breithaupt 2011; Weissburg 2011). Aquatic crustaceans live in a world full of chemicals. Therefore, major tasks for chemosensory systems are detecting and discriminating mixtures of odors against highly variable backgrounds. The relationship of odor-guided behaviors, orientation strategies, plume structure and dispersal, and morphological aspects of the olfactory system has been intensely analyzed in a variety of generalist decapod crustaceans such as brachyuran crabs (e.g., Weissburg and Zimmer-Faust 1994), crayfish (Moore and Grills 1999), spiny lobsters (Horner 2004) and peracarid crustaceans (Premke et al. 2003; Krång and Baden 2004; Ide et al. 2006). These studies picture a highly complex dependence of flow physics, odor reception, stimulus processing and locomotory responses. Abiotic factors such as the

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properties of chemicals, water flow and plume structure, but also biotic factors such as animal size, sensor span (i.e., distance between two antennae) and the underlying neuronal circuitry of the peripheral and central olfactory pathways are only a few of the key parameters shaping olfactory-guided behaviors. Most Malacostraca studied so far are well adapted to master the challenges of olfaction. The unimodal olfactory sensilla on the first pair of antennae called aesthetascs and bimodal chemo- and mechanosensory sensilla, as well as associated brain centers are of particular importance for long-distance chemoreception (Hallberg and Skog 2011). As our present knowledge of olfactory-guided behaviors is primarily founded on studies in Decapoda, we are only beginning to explore chemoreceptive foraging in other crustacean species (e.g., Premke et al. 2003; Ide et al. 2006; Tiselius et al. 2013). This study sets out to expand our knowledge in this field beyond the classical crustacean model organisms. We analyzed the ability of the omnivorous isopod *Saduria entomon* (Valvifera) to localize a broad range of waterborne stimuli using a custom made Y-maze bioassay. In previous studies, it was shown that *S. entomon* possesses both the sensory (Pynnönen 1985) and central nervous equipment (Kenning and Harzsch 2013) required for receiving and processing olfactory stimuli. In order to document the efficiency of our setup, we used the same bioassay to examine olfactory-guided behavior in the marbled crayfish *Procambarus fallax* forma *virginalis* (Decapoda). It is well documented that crayfish use chemical senses to detect the presence of predators, assess social hierarchies and find shelters and food (see Breithaupt 2011). They also display a highly effective odor-tracking behavior in Y-mazes (Schneider et al. 1999).

Materials and methods

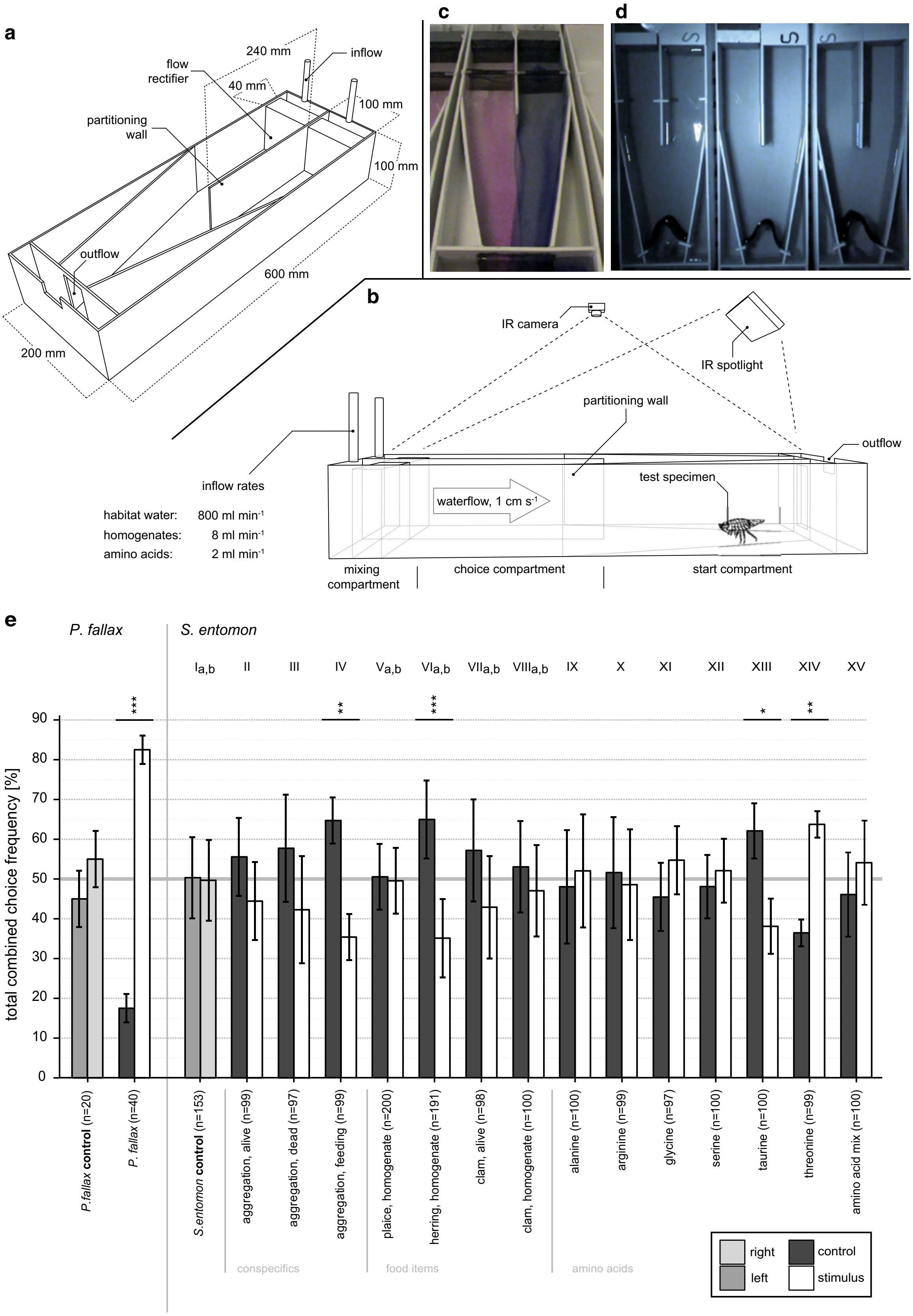
Animals

Stocks of *Saduria entomon* Linnaeus 1758 were collected in May 2013 with a dredge at the bottom of the Tvärminne Storfjärd, Gulf of Finland (~50 m, 59°50′N, 23°15′E). Animals of different sizes and both sexes were kept food deprived and without light in several aquaria with a constant supply of filtered seawater (~6 PSU) at 6 °C for 2½ weeks prior to testing. To minimize cannibalism within the stock, animals were broadly grouped into four size classes (very large, $\geq \sim 5$ cm; large, 3–5 cm; small, 1–3 cm; very small, $\leq \sim 1$ cm) and kept separately. Specimens of *Procambarus fallax*, Hagen 1870 forma *virginalis* (Martin et al. 2010)—hereafter only called *P. fallax*—originated from a colony in the Department of Cytology and Evolutionary Biology in Greifswald. Animals of different sizes were kept food deprived for 1 week in aquaria filled with fresh water at 18 °C before being tested (Seitz et al. 2005).

Fig. 1 Experimental setup and overview of experiments. **a**, **b** Blueprints of the Y-maze seen from different perspectives. Inflow nozzle from peristaltic pump not shown. **c** Calibrating the water flow using methylene red and standard pen ink. Note that the nets were removed prior to experiments. **d** Night vision shot of stimulus treatment experiment. Only three of four setups are shown. **e** Overview of control and odor treatment experiments. Note that the combined relative choice frequency and standard deviation from all four parallels are shown. There are no significant differences between the two branches of the Y-maze in the negative-control experiments for both, *Saduria entomon* and *Procambarus fallax*. *P. fallax* showed a highly significant preference for the odor. Only four experiments with *S. entomon* yielded a significant response. The combined stimulus (IV), herring (VI_{a+b}) and the amino acid taurine (XIII) had a significantly repellent effect, and only in one experiment (XIV, threonine) the stimulus was attractive (see text for details). Random chance (50 %, based on the area of branches vs. total area of the maze) is indicated by the *gray solid line*. *Asterisks* denote significance levels (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) ▶

Bioassay

Laboratory experiments on *S. entomon* were conducted at the Tvärminne Zoological Station, Finland, using four identical replicate Y-maze setups made of PVC (Fig. 1a–d). These bioassays were composed of an inflow/mixing compartment followed by a flow rectifier, a choice compartment partly separated by a partitioning wall forming the branches, the start/acclimatization compartment and an outflow section (Fig. 1a, b). A gravity-fed water flow system provided a continuous supply of filtered but otherwise untreated habitat water of about 800 ml min^{-1}, yielding a quasi-laminar flow with a speed of ca. 1 cm s^{-1} at the downstream end without formation of visible turbulences. Retention time of the water was calculated at 20 min. All experiments were performed in a climate chamber at 6 °C in darkness, observed using night vision goggles and recorded using an infrared spotlight and camera (Sony DCR-HC47; Fig. 1d). Although the experiments were designed to focus on olfactory-mediated behaviors by excluding optical stimuli, four experiments were conducted a second time with artificial light (Philips Softone 12 W Energy Saver, ~1.0 m above Y-maze, intensity: 1 µE m^{-2} s^{-1} at the level of the animal). Per trial and assay, a single animal was left to acclimatize at the downstream end of the channel for 5–10 min while partly exposed to the stimulus before being released (compare flow visualization in Fig. 1c). A total of 1800 trials using 14 different stimuli that this animal is likely to encounter in its natural habitat were performed (see next section and supplementary material I). A valid choice was recorded when the animal passed half of the partitioning wall. Based


a
240 mm
inflow
flow rectifier
40 mm
100 mm
partitioning wall
100 mm
outflow
600 mm
200 mm
c
d
b
IR camera
IR spotlight
partitioning wall
outflow
test specimen
inflow rates
waterflow, 1 cm s-1
habitat water: 800 ml min-1
homogenates: 8 ml min-1
amino acids: 2 ml min-1
mixing compartment
choice compartment
start compartment
e
P. fallax
S. entomon
Ia,b
II
III
IV
Va,b
VIa,b
VIIa,b
VIIIa,b
IX
X
XI
XII
XIII
XIV
XV
***
**
***
*
**
total combined choice frequency [%]
0
10
20
30
40
50
60
70
80
90
P.fallax control (n=20)
P. fallax (n=40)
S.entomon control (n=153)
aggregation, alive (n=99)
aggregation, dead (n=97)
aggregation, feeding (n=99)
plaice, homogenate (n=200)
herring, homogenate (n=191)
clam, alive (n=98)
clam, homogenate (n=100)
alanine (n=100)
arginine (n=99)
glycine (n=97)
serine (n=100)
taurine (n=100)
threonine (n=99)
amino acid mix (n=100)
conspecifics
food items
amino acids
right
left
control
stimulus

on observations in a pilot study, the maximum duration of a single trial was set to 5 min. If by then no choice was made, the trial was stopped, counted as invalid and excluded from the analysis (see supplementary material I, note the high ratio of valid choices). Test individuals were used only once per treatment, and in all experiments except for experiment II (see below), all size classes were considered. Experiments on *P. fallax* were performed at the Department of Cytology and Evolutionary Biology, Greifswald, using the same Y-maze assay and flow parameters. A total of 60 trials were conducted at natural daylight to test the responses to aqueous extracts of an artificial food item.

Stimuli

We performed 15 experiments with *S. entomon* in four (in experiments VII and VIII two) replicate Y-maze aquaria simultaneously. Per trial, a single stimulus was applied randomly to the left or right side of the mixing compartment, while the other branch remained empty (control, application of seawater without stimulus). For the application of homogenates, we used a peristaltic pump at a flow rate of ~ 8 ml min^{-1} and amino acid solutions were applied at 2 ml min^{-1}.

Negative control

No chemical stimuli were applied. In experiments $I_{a,b}$, a total of 160 control trials were conducted at both light conditions to account for a possible left- and right-side bias. Equally, 20 control trials were performed on *P. fallax* at both light conditions.

Conspecifics

During winter, younger cohorts of *S. entomon* migrate into shallower waters (Nikitina and Spasskii 1963). The reason for this migration is unknown, but as they are effectively avoiding aggregations of larger specimens, it may be linked to reducing cannibalism in a time of scarce resources. In experiment II, we tested the response of small and very small specimens when encountering larger conspecifics. Thus, large and very large animals ($n = 6$) were caged in the mixing compartment and concealed by the flow rectifier but otherwise were left untreated.

Experiment III was designed to mimic the “smell of death” as a repellent stimulus (Kats and Dill 1998; Hazlett 2011). We tested for the reaction when confronted with aggregations of recently deceased animals placed in the mixing section ($n = 8$, dissected less than 30 min prior to experiment). To facilitate the release of metabolites, carcasses were crushed. Yet, as *S. entomon* also preys on conspecifics (Leonardsson 1991), this stimulus may also have an attractive quality.

In experiment IV, we tested a combined stimulus of aggregated animals and a potential food item. Fresh filets containing muscle and skin tissue of European plaice (*Pleuronectes platessa*) was offered to a group of *S. entomon* ($n = 6$) also caged in the mixing section (compare experiment II). The caged animals immediately started feeding on the dead fish violently, confirming our assumption that they were sufficiently deprived and motivated to locate food-related odors.

Food items

In experiments $V_{a,b}$ and $VI_{a,b}$, pieces of plaice muscle tissue and herring (*Clupea harengus*), respectively, were homogenized, filtered and applied with a peristaltic pump. Experiments were conducted at both light conditions.

Baltic clam (*Macoma balthica*) which is known to be a major dietary component of *S. entomon* (Ejdung and Elmgren 2001), was used in four experiments, alive and as a fluid homogenate at both light conditions ($VII_{a,b}$ and $VIII_{a,b}$).

Amino acids

In experiments IX–XV, we tested the response to the amino acids alanine, arginine, glycine, serine, taurine and threonine (Ide et al. 2006). Based on the background concentration of amino acids of ca. 2×10^{-7} mol l^{-1} in the Baltic Sea (see Mopper and Dawson 1986 and references therein), aqueous solutions were applied separately and as a mixture at an in-flow concentration of 1×10^{-5} mol l^{-1} using a peristaltic pump.

Stimulus for P. fallax

An aqueous extract of TetraWafer-Mix, a staple food mix for carnivorous and herbivorous bottom-feeding fish and crustaceans which has proven to be quite attractive for *P. fallax*, was used to test chemotaxis in the Y-maze bioassay.

Statistical analysis

Choices from all trials per replicate and experiment were combined to a choice frequency per replicate and experiment, and subsequently pooled, yielding a combined choice frequency for each experiment (absolute counts; see supplementary material II). These data were evaluated for differences in stimulus versus control treatment using Chi-squared goodness-of-fit test (χ^2) in R (see supplementary material III, version 3.1.1, Team et al. 2005). We tested for a left–right bias in the control experiments, for differences between stimulus and control branches in the odor treatment experiments, and—where conducted—for differences in light treatments.

Results

Negative control

Figure 1e summarizes all results for the negative-control and stimulus treatment experiments. Out of 160 negative-control trails carried out with *S. entomon*, 153 valid trials showed that there was no preference for one branch given the lack of significance (experiment I_{a+b}: $\chi^2 = 0.065$, $P = 0.936$, $n = 153$). Likewise, no statistically significant preference for one branch was found in the experiments with *P. fallax* ($\chi^2 = 0.2$, $P = 0.65$, $n = 20$).

Odor treatments

When testing *P. fallax*, 82.5 % of the animals were attracted to the stimulus ($\chi^2 = 16.9$, $P = 3.94 \times 10^{-5}$, $n = 40$). We take this as evidence that in principle the design of our bioassay is suited to analyze odor preferences in malacostracan crustaceans.

Conspecifics

No significant response from *S. entomon* was observed when confronted with aggregations of living conspecifics (experiment II). A similar reaction is seen in the "smell of death" experiment (III, conspecifics dead). Although the difference between stimulus and control branch seems more distinct, Chi-squared test yielded no significance. In the combined stimulus experiment (IV) in which conspecifics fed on plaice, a highly significant response toward the control was provoked ($\chi^2 = 8.49$, $P = 0.004$, $n = 99$). Interestingly, neither the aggregation of animals (II) nor the fish itself (V_{a+b}, see next section) caused a comparable response.

Food items

Muscle tissue homogenate from plaice (experiment V_{a+b}) was the least effective treatment, showing a random distribution of choices. The strongest response of all experiments with *S. entomon* was observed when exposed to a homogenate of herring muscle tissue (experiment VI_{a+b}). Again, the animals significantly chose the control ($\chi^2 = 17.01$, $P = 3.7 \times 10^{-5}$, $n = 191$). *Saduria entomon* showed a barely distinct response toward the control branch when encountering exudates from living clam as well as homogenates of this clam (experiments VII_{a+b} and $VIII_{a+b}$). However, none of these stimuli elicited a significant response.

Amino acids

With the exception of taurine and threonine, there were no further significant responses in testing single amino acids or the amino acid mixture. Exposure to taurine provoked a significant selection of the control compartment (XIII; $\chi^2 = 5.76$, $P = 0.016$, $n = 100$). Threonine was the only stimulus that was significantly attractive (XIV; $\chi^2 = 7.36$, $P = 0.007$, $n = 99$).

Influence of light conditions

In one of four experiments that were conducted a second time with artificial light, we found an influence of the ambient light conditions on the behavior of *S. entomon*. In experiment VI, *S. entomon* significantly preferred the control (VI_a; $\chi^2 = 17.69$, $P = 2.6 \times 10^{-5}$, $n = 96$) to the stimulus (VI_b; $\chi^2 = 2.67$, $P = 0.10$, $n = 95$).

Discussion

Our findings on *Procambarus fallax* support data from previous studies that crayfish can effectively discriminate and localize odors with high precision (see Breithaupt 2011). We take this as evidence that in principle the design of our bioassay and the experimental parameters are suitable to study odor tracking in malacostracan crustaceans. In 1800 trials using the same bioassay, *Saduria entomon* was attracted only to the amino acid threonine and displayed a significantly aversive behavior to taurine, herring and feeding conspecifics. The fact that these animals were not attracted to food-related stimuli is puzzling. Several not mutually exclusive hypotheses might explain these results: (1) Apart from threonine, the range and/or concentrations of stimuli tested did not act as an attractive compound for *S. entomon*; (2) "long-distance" chemoreception as tested in our bioassay is not ecologically relevant for these animals, and a directed orientation toward food-related odor sources is not part of their foraging strategy; (3) environments that are spatially (and/or temporally) restrictive do not represent an ecologically relevant situation for *S. entomon* and hinder a successful tracking behavior.

Evaluating experimental parameters: water flow, light regime, active compounds and concentrations

In ecophysiologically relevant experiments, test specimens have to be exposed to stimuli that resemble the conditions in their natural habitat in terms of quality, quantity and mode of administration. With regard to flow conditions, velocities below or above a certain threshold will impede or even prevent a successful localization of odor sources (Moore and Grills 1999). The Tvärminne Storfjärd surface water flow velocities have been calculated at 2–4 cm s^{-1} with moderate annual variations (Alenius et al. 1998). Unfortunately, no information on bottom water currents is available, but net flow can be expected to be in the same range. Although *S. entomon* showed significant responses to four of the applied stimuli, we nevertheless believe that the role of water flow must be explored in more detail in future experiments.

The eyes of *S. entomon* possess a high sensitivity with maximum absorption close to the wavelength of maximum light transmittance in their habitat (Lindström et al. 1991), which, however, features rather low ambient light conditions (Lindström 2000). Poor light conditions in the habitat, dorsal position of the eyes and relatively small optic neuropils underlying the eyes (Kenning and Harzsch 2013) argue against vision being an important factor in foraging. However, similar to other environmental factors (Pynnönen 1996; Johansson 1999), our results suggest that light may have a distinctive influence on the behavior of *S. entomon* and further attention should be paid to the aspect of light-induced modulations of behavior.

With respect to the stimulus itself, it has been suggested that certain chemicals represent an inappropriate cue because of little correlation to prey availability. In an omnivorous scavenger such as *S. entomon*, however, such concerns may be less important as it can be assumed that almost any carrion is suitable as food. In contrast, unnaturally high concentrations of a stimulus may have a more profound impact and induce behaviors other than tracking a stimulus source (Weissburg et al. 2002). In other malacostracan crustaceans, homogenates, extracts and soaks of prey were shown to be particularly prone to overdosage, producing unrealistic high stimulus intensities (Weissburg et al. 2002). The responses of mixtures and aqueous prey extracts can be strongly concentration dependent, showing a synergistic suppression effect at higher concentrations (Carr and Derby 1986). Although amino acids were shown to act as an attractive cue at concentrations four orders of magnitude above their background concentration in amphipods (Ide et al. 2006), the response elicited by free amino acids and mixtures is particularly dose dependent (Carr and Derby 1986). These facts offer one possible explanation why most of the amino acid solutions we used in the current study, including the mixture, failed to generate a response. Future tests inevitably have to consider dose-dependent effects.

The two amino acids that did evoke a significant response further complicate the picture. The only attractive odorant in our test (threonine) has not been reported to be a particularly active compound for other foraging crustaceans. In contrast, taurine is well known to initiate food searching and feeding behaviors in Malacostraca (Carr and Derby 1986) and is regarded as one of the predominant amino acids released from injured or freshly dead invertebrates (see Webb and Johannes 1967 and references therein). The finding that *S. entomon* avoids this amino acid raises the question whether concentration dependency due to overstimulation may cause this effect or whether taurine may also be an active compound indicating the presence of, e.g., a predator. Living animals release a wide range of different compounds and metabolites (e.g., Simpson et al. 1959; Zimmer et al. 1999), and it is well known that many species use chemical cues from potential predators (i.e., kairomones) for alarm responses (e.g., Hesselschwerdt et al. 2009).

Evaluating experimental designs: Is directed orientation toward food-related odor sources part of *Saduria entomon's* foraging strategy?

Studies on the morphology of the antennae as well as the underlying neuronal network suggest that *S. entomon* is able to perceive, process and use odors (Pynnönen 1985; Kenning and Harzsch 2013). Ablation experiments by Pynnönen (1985) showed that the aesthetasc equipment of antenna 1 is necessary for *S. entomon* to react to chemical stimuli. However, the results presented and discussed above challenge our understanding of foraging strategies of scavenging crustaceans. Green (1957) analyzed the grasping and feeding mechanism of *S. entomon* and provided several observations suggesting that like in terrestrial isopods, (chemo)tactile senses are more important in finding food than olfaction. Reports by Narver (1968) and Pynnönen (1985) also suggest that foraging behavior in *S. entomon* may rather be stimulated by olfactory cues than actually be guided by olfaction. Unpublished observations by the authors using a different bioassay indicate that although baited traps set out in a more spacious environment frequently are crawling with specimens of *S. entomon*, the animal's tracks toward the bait are seemingly random, including large zigzagging loops rather than on a direct course (Kenning et al. in prep). The behavior shown by *S. entomon* thus rather corresponds to kinesis than to actual taxis. The experimental design did not account for this possibility and thus proved to be a suboptimal approach to studying olfactory-guided behaviors in this species. Moreover, it illustrates that established methods to study behavior cannot be readily transferred to other crustacean species.

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Publikation III

Kenning M, Lindström M, Harzsch S (eingereicht)

Randomness on the trial – Food search patterns in a marine Isopod

Randomness on the trial –
Food search patterns in a marine Isopod

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Keywords

Saduria entomon, Malacostraca, olfaction, odor tracking, behavior, bioassay

Abstract

We studied food search patterns in *Saduria entomon* using a 3,600 cm² quadratic arena. Although distinct differences in various movement parameter exist between control and stimulus treatment, *S. entomon* does not show a recognizable search pattern. Ablation of both, antenna one or two, resulted in an almost complete inability to locate a stimulus. A statistical orientation test suggests chemotaxis superimposed on positive a rheotaxis as the elementary orientation mechanism when antennae are intact. However, random elements in the search strategy cannot be ruled out. When antennae are dysfunctional, orientation test suggests kinesis or a true random walk in combination with distinct upstream migration. The results are discussed with regard to the sensory and neuronal basis of olfaction.

1. Introduction

Living in a world full of chemicals, marine animals evolved elaborate chemosensory systems in order to find food and shelters, to communicate, as well as to assess the presence of predators or potential mates. Malacostracan crustaceans, decapods in particular, are a paragon of a chemical detector. Equipped with numerous chemosensory sensilla located on virtually every part of the body (Derby & Steullet, 2001; Hallberg & Skog, 2011, Schmidt & Mellon 2011, Derby & Weisburg 2014) and by applying specific behaviors like "sniffing" (i.e. flicking of their antennae; reviewed in Koehl 2011), as well as odor tracking, many species display a high degree of precision and fidelity in detecting, differentiating, and localizing waterborne chemicals. Consequently, some of them achieved model status in various disciplines like in chemical ecology and the study of chemical senses (see e.g. Duffy & Thiel 2007, Derby & Thiel 2014). For many other crustaceans however, as Weissburg (2011) recently stated: "One wonders whether crustaceans that are [...] small relative to the plume [...] might employ similar strategies [as insects], but we have yet to examine closely the behavior of animals in this situation". Clearly, there is still a number of open questions. Weissburg's thoughts arise from the rationale that the size of an animal, and hence of its sensory array, may have a considerable effect on its capability to detect and precisely locate an odor source. Given the complex dependencies of size, scale and relative motions in fluid media, i.e. the framework set by Reynolds-, Prandtl- and Péclet numbers (reviewed in e.g. Weissburg 2000; Koehl 2006, Koehl 2011, Weissburg 2011), small animals are thought to face particular difficulties in detecting navigational useful concentration asymmetries in an odor plume, crucial for localizing its source.

Most earlier detailed ethological studies were on crustacean species that are relatively large with regard to an odor plume emitted by e.g. a prey item (e.g. spiny lobster, lobster or crayfish; Tierney & Atema 1988, Moore et al. 1991, Weissburg 2003) and standardized behavioral assays to analyze odor tracking behaviors were established. The mechanisms and ecological consequences of the chemosensory proficiency in these species are derived from investigations on social or predator-prey interactions. Consequently, our knowledge on food searching strategies shows a considerable bias, neglecting smaller pelagic and benthic species like grazer or scavengers.

In previous studies it was shown that the scavenging marine isopod *Saduria entomon* LINNAEUS 1758 possesses both the sensory (Pynnönen 1985) and central nervous equipment (Kenning & Harzsch 2013) required for detecting and identifying olfactory stimuli. Ablation experiments indicated that the chemosensory equipment of the first antenna is a prerequisite for *S. entomon* to respond to waterborne chemicals (Pynnönen 1985). However, observations by Kenning et al. (2015) using a Y-maze bioassay documented that *S. entomon* reacted to homogenates of fish and conspecifics in an entirely unexpected way. In experiments in which crayfish localized food related odors with high fidelity, *S. entomon* performed with low precision in locating an odor source which otherwise is found to be attractive. Unattested observations by scientific divers at the Tvärminne Zoological Station in Finland as well as a report by Narver (1968) indicated that *S. entomon*s' movements in the natural habitat are rather undirected, random, and very unlike “typical” olfactory-guided foraging behavior as seen in decapods (see Derby & Weissburg 2014 for a review). Therefore, Kenning et al. (2015) suggested that foraging behavior in *S. entomon* may be stimulated by, rather than actually guided by olfaction. Moreover, the aforementioned study demonstrated that methods established to study odor guided behaviors in large decapods crustaceans represent a suboptimal approach in this species, illustrating that certain behavioral tests cannot be readily transferred from one species to another. Therefore, this study sets out to test if a newly designed bioassay can provide new insights into odor guided behaviors in this isopod species. Here, we examined the food search behavior of *S. entomon* using a 3.600 cm^2 quadratic flow through arena (hereafter called the squarena). Towards an understanding of the general movement patterns of the animal, we performed controls and documented various movement parameters of the animals' walking without reference to a potential target, providing a reference frame for future studies. We also tested the responses of *S. entomon* to pieces or whole specimen of smelt (*Osmerus eperlanus)*. To assess the importance of functional antennal sensilla in localizing food, we performed bilateral chemical ablations of the first and second antenna, respectively. Along these lines, we address the question if these animals are actually able to *locate* an odor source by chemotaxis, or by using (chemo-)tactile senses similar to their terrestrial relatives.

2. Material and Methods

2.1 Animals

The arctic relict species *Saduria entomon* is commonly found in brackish waters of the eastern and northern Baltic Sea, several European as well as North American freshwater lakes. It is common from 25 m down to 290 m (Haahtela 1990), but younger cohorts are often found in shallower waters (Nikitina & Spasskii 1963, Zmudzinski 1966). Males may reach a size of up to 8 cm, females are generally smaller. As an opportunistic and omnivorous scavenger, *S. entomon* feeds on dead fish and other carcasses but also on smaller conspecifics, amphipods (e.g. *Monoporeia affinis)* and clams (*Macoma balthica*; Green 1957, Ejdung & Elmgren 2001). *S. entomon* is classified as nocturnal (Westin & Aneer 1987), but the animal can be observed foraging both day and night, without obvious differences in its activity. Test specimens were obtained from the bottom of the Tvärminne Storfjärd, Gulf of Finland (~ 60 m, 59° 51' N, 23° 16' E) in May 2014 using a bottom trawl. Animals of various sizes and both sexes were kept without food and in darkness (DD:24) in aquaria with constant supply of filtered seawater (~ 6 PSU) at 6°C for 2 ½ weeks prior to testing. The animals were roughly grouped into four size classes (very small, small, large, and very large) and kept separately in order to minimize predation within the stock.

2.2 Bioassay

Experiments were conducted at the Tvärminne Zoological Station in Finland using a custom 3600 cm² squarena (Fig.1) made from PVC (walls) and semi translucent acrylic glass (bottom). All experiments were performed in a climate chamber at ~ 6 °C in darkness. A gravity driven water supply provided a continuous inflow of 200 $\text{ml}\cdot\text{min}^{-1}$. Given water level of 8 cm, the effective volume of the squarena was 28.8 l, resulting in a net retention time of 144 min. Dye tests showed that a stimulus released at the inflow dispersed considerably and reached the outflow within 10 minutes in a rather broad plume. Infra red illumination was provided by an IR spotlight installed beneath the arena. A modified webcam (Logitech HD C615, infra red filter removed) installed 1.5 m above the apparatus allowed observation and recording of the experiments. Prior to an experiment, a piece of *O. eperlanus* was offered to the designated test specimen in order to attest its motivation for finding food. Per experiment, a single animal was left to acclimatize for at least 15 minutes at the downstream end of the squarena before being released. Preliminary studies have shown that the animals would stop exploring the mesh separated start compartment within 5 to 10 minutes. The maximum duration of a single experiment was 10 min. After each experiment the arena was emptied and refilled to prevent odor oversaturation of the water and/or habituation of the next test specimen during the acclimatization process. Test individuals were used only once and both sexes as well as all size classes were considered. We performed a total of 36 experiments with *S. entomon* in three different experimental conditions:

Controls

Like in the ablation and stimulus experiments (see below), all animals were immobilized in the same manner but no antennae were inactivated. No stimulus was applied. The center of the arena however, was defined as the target site in order to compare movement parameters.

Stimulus treatment

A piece or a whole small specimen of smelt (target) was randomly placed near the center of the squarena. To ensure comparability, all animals were immobilized in the same manner as ablation animals (see below) for at least five minutes.

Ablation experiments

Ablations were performed at least 3 h prior to the start of an experiment. The animals were immobilized half submersed in a styrofoam bowl with constant supply of aerated habitat-water. A glass capillary filled with distilled water was placed over the respective antennae for 20 minutes and sealed with vaseline. In decapods crustaceans in was shown that exposure to distilled water osmotically disrupts the outer dentritic segments of chemosensory neurons, deactivating the receptor for several hours to days (Gleeson et al. 1996, Steullet et al. 2001). The function of some mechanosensory neurons however, is retained (Derby & Atema 1982). After removal of the capillary, the animals were left to recover in a separate aquarium. The target (smelt) was placed randomly near the center / upstream end of the squarena.

2.4 Movement analysis, evaluation of performance and statistical analysis

Saduria entomon's behavior was recorded with a speed of five to ten frames per second and subsequently processed with the video analysis software Tracker (Brown 2015). Although *S. entomon* swims occasionally and quite efficiently, crawling is the preferred mode of locomotion. Hence, only XY coordinates and corresponding time frames with the head as the reference point were recorded (Fig.1). For further analysis and in order to optimize signal/noise ratio as well as ensure comparability, a fixed time step of 1 second was used. From the resulting coordinates, we compiled path- and heat-maps, and calculated additional variables to describe the animals' movement using the software R (R Development Core Team 2008); i.e. step size L_i, speed V_i, total distance traveled L_t, distance to the target D_t, turning angle θ_i, concentration parameter ρ, angular deviation S, path tortuosity T). The heading angle (α) alias the directional error was defined as the deviation between the current movement vector from a straight line towards the target (stimulus) and was calculated as -180° to 180° with a positive value for the target on the right side. For the analysis however, we used absolute angles (i.e. positive values). Additionally, we calculated the directional error and distance with regard to the water inflow. An animal's path can be described as a sequence of steps of varying size and corresponding directional changes between these steps. In contrast to Brownian motion which comprises uniformly distributed turns (all turning angles being equally likely thus producing a true random walk), animals show a tendency to continue the movement in "the same" direction. As turning/heading angles are distributed in a circular analogue of the normal distribution, they can be described by two parameters: the circular mean and a concentration parameter ρ, taking values between 0 and 1 (i.e. the amount of "the same"). With decreasing ρ, turning angles converges to a uniform distribution. With increasing ρ, they approach a normal distribution centered around the mean. Thus, ρ serves a measure of directional persistence of the movement, and, given a non-uniform distribution, the mean as a measure of overall directionality. Theoretically, an animal walking directly towards a given target would then feature a rather high concentration parameter of heading and turning angles and a directional error of only a few degrees. On the other hand, an animal walking without a particular directedness towards a target would show a bidirectional distribution of heading angles around ± 90° and a rather low concentration parameter. The concentration parameter and angular deviations were calculated according to Batschelet (1965). We defined performance as the ability to locate the target within a given time. Thus, search efficiency *ε* was defined as the ratio of success and the log transformed product of distance traveled to target and time spent searching. In order to provide a more universal efficiency assessment, we calculated the tortuosity *T* alias the straightness index of the animal's paths using the ratio of distance from starting point to final position and total distance travelled (Batschelet 1981). The tortuosity of a path essentially is inversely related to involved orientation mechanisms with the best mechanism allowing the animal to reach its target along a straight line, featuring a tortuosity of 1. One-way-ANOVA and post-hoc all pairwise multiple comparison corrected for Holm-Bonferroni were used to test for differences of performance parameters (e.g. efficiency, time to target, tortuosity) between experimental designs and/or single experiments. Time series were log-transformed before being analyzed. We evaluated the direction and strength of the relationship of respective parameter *versus* target distance using Pearson's correlation test for speed, and the circular-linear correlation test proposed by Mardia (1975) for angular data. We disregarded using distances = 0 as parameters tested would incorporate a considerable bias with the animal directly at the target. An orientation test as proposed by Benhamou & Bovet (1992) was used to characterize the orientation mechanism displayed by *S. entomon*. The distribution of side-dependent turning angles was calculated and the mean evaluated using one-tailed t-test. In the case of kinesis or any other undirected movement, the animal's (side-dependent) turns are normally distributed without reference to the preceding turn or target direction, the mean thus does not differ significantly from 0. In the case of taxis, the animal will turn right when the preceding turn was oriented to the left of the target and vice versa. Hence, the mean will be significantly higher than 0.

3. Results

3.1 Controls

Table 1 summarizes the movement parameters for all experiments. With the exception of two trials, all control animals constantly moved and explored the squarena, illustrated by the largely homogeneous path- and density distribution, showing that the whole squarena has been visited (Fig.3A,B). The walls and upstream corners however, feature the highest densities. Control animals displayed the highest average movement speed of 21.4 ± 14.3 $mm\cdot s^{-}1$, ANOVA $p < 0.001$, Fig.2A,3C,G), and covered distances between 1.2 m and 19.9 m in 10 minutes (on average 12.0 ± 6.5 m). The animal's paths are rarely straight but rather smooth protracted curves (compare Fig.3D). The turning angles show a high degree of persistence, being the smallest of all experimental groups with a moderate variation among the population (on average $\theta = 0.56 \pm 40.0$ °, $\theta\rho = 0.75$; Fig. 2B, 3E,H). Tortuosity was calculated with 0.03 ± 0.03. Heading angles are bidirectionally distributed, missing a particular directedness ($\alpha = 64.2 \pm 78.6°$, $\alpha\rho = 0.06$; Fig. 2C, 3F,I). With respect to inflow distance there is no apparent influence on speed or turning angles. In contrast, corresponding heading angles show a considerable decline with decreasing distance to the inflow (Fig.7A). However, comparison of side dependent turning angles and its distribution reveals that the animals walked with reference to neither the squarena center nor the water inflow (orientation test $p > 0.05$). Interestingly, when animals circuited the squarena, most of them did so in a counter clockwise direction, indicated by the higher density of negative heading angles (Fig. 3F).

3.2 Stimulus treatment

According to our criteria for orientation and/or a successful navigation, only a single trial was rejected due to an absolute lack of any movement of the respective animal. Thus, nine out of ten animals were able to locate the target within the given time (mean time to target: 135 ± 164 s; Fig.4A). The animals covered distances between 0.8 m and 10.7 m (on average 2.8 ± 3.3 m) and displayed an average movement speed of 14.4 ± 14.0 $\mathrm{mm \cdot s^{-1}}$. With decreasing target distance, there was a considerable decline in mean movement speed, especially within the last 100 mm, which is not exclusively attributable to animals stopping at the target (Fig.2A,4C,G). Compared to control trials, the animals paths were slightly more contorted and irregular (θ = 0.66 ± 58.1°, $\theta\rho$ = 0.46; Fig. 4B, E, H). Stimulus treated animals showed the second highest angular deviations and second lowest concentration parameter coefficients of turning angles, expressing a wider distribution of turning angles as compared to the control. Turning angles were negatively, heading angles positively correlated with target distance, although correlation strength is very low for both parameters (not shown). We found a rather high straightness index of T = 0.44 ± 0.32, with mean heading angles constantly converging to a smaller directional error (α = 52.9 ± 70.5°, $\alpha\rho$ = 0.24; Fig. 4F, I). In the immediate vicinity of the target (i.e. around 50 mm) however, heading angles increase again, indicating of a new state of arousal. Untreated animals showed best performance with regard to time spent searching and distance travelled (ANOVA p = < 0.01 for stimulus vs. ablations, p < 0.001 for stimulus vs. control; see Tab. 1). Orientation test strongly suggests a tactic orientation of the animals towards the target (one-tailed t-test p<0.001). It is noteworthy that in two cases the orientation test was insignificant and thus suggested kinesis. Interestingly, with regard to the water inflow, orientation test was marginally insignificant (p = 0.052). Also, we did not identify a significant influence of water inflow on the other movement parameter tested (Fig.7B).

3.3 Ablation experiments

Chemical ablation of antenna 1 or antenna 2 resulted in the almost complete inability to localize a target within the given time frame, illustrated by the low efficiency scores ε (Table 1).

Ablation of antenna 1

Only three of nine animals were able to locate the target (time to target: 328 ± 16 s). Path- and density plots (Fig.5A, B) display highly contorted and zigzagging paths including several stops and rather large directional changes, much more irregular than in the stimulus treatment, resulting in the second largest turning angles and considerable variation among the population (θ = 0.86 ± 59.3°, $\theta\rho$ = 0.46, Fig. 5E, H). Antenna one ablated animals were the slowest, displaying an average movement speed of 10.4 ± 12.7 $\mathrm{mm \cdot s^{-1}}$ (ANOVA p < 0.01, Fig. 2A, 5C, G) with a slight acceleration with decreasing target distance. Tortuosity of paths was calculated as T = 0.13 ± 0.14 for the population mean. Interestingly, we again find a high location density at the left upstream, and also at the left downstream corner. In general, it appears that once an animal hits a wall it uses that wall to guide its movement, coming to a halt or trying to crawl onto it when encountering a corner. Thus, the center of the squarena is rarely crossed. Surprisingly, the animals feature the lowest angular mean for the directional error (α = 24.9±78.9°). But the concentration parameter ($\alpha\rho$ = 0.05) and Fig. 5F clearly demonstrate a multidirectional distribution, strongly suggesting a lack of directedness. Correlation coefficients of speed, turning angles and directional error with respect to target distance are significant but consistently very low. Binomial regression illustrates that the animal shows an average directional error of close to 90° (Fig.5I). At a distance of about 150 mm to the target, angles are dispersing; with the three successful animals driving the directional error towards lower values (excluding successful trials eliminates this bias, not shown). Interestingly, some animals were closing in on the target but apparently failed to recognize it, eventually passing it in a distance of only a few millimeters (Fig. 5 D). Similar to the stimulus experiments, heading angles (of successful animals) increase again at distances of about 50 mm to the target. Like in the control group, orientation tests indicate that the animals oriented without reference to the target and used kinesis as the elementary orientation mechanism (orientation test $p > 0.05$). However, the results also suggest a positive rheotactic movement towards the inflow ($p < 0.05$). Movement speed increases, turning and heading angles decrease with the animal approaching the water inflow (Fig.7C).

Ablation of antenna 2

Like in the experiment described above, only three of nine animals were able to locate the target (time to target: 282 ± 71 s). However, the curves of the animals paths are much smoother compared to the ablation of antenna 1, and although we again find the walls and the left upstream corner highly attractive, the whole squarena has been searched. Movement speed showed a high but constant variation with decreasing target distance. Deceleration in close vicinity to the target as seen in the other experiments is not present (Fig.2A, 6G). Turning angles are the highest in all treatment experiments ($\theta = 1.29 \pm 53.1°$, $\theta\rho = 0.61$, Fig. 6E,H) showing no variation with regard to target distance. Heading angles were multidirectionally distributed, with its mean at a constant level with decreasing target distance, indicating a lack of directedness ($\alpha = 72.44 \pm 78.86$, $\alpha\rho = 0.05$; Fig. 6F, I). Also, the change in the angular distribution in close vicinity to a target was not as abrupt as seen in the other two experiments. The three successful animals drove the directional error towards lower values but excluding these trials eliminates this trend (not shown). Consequently, path tortuosity was again very low ($T = 0.09 \pm 0.05$). An orientation test with the target as reference suggests kinesis as orientation mechanism (one-tailed t-test $p > 0.05$). However, like in the experiment described above, the results also indicate a positive rheotactic movement towards the inflow ($p < 0.05$; see alsoFig7D).

The average time spent searching for the target did not differ significantly between stimulus and ablation experiments (ANOVA $p > 0.05$). However, in consideration of the few successful trials in both ablation experiments, a larger number of trials likely would reveal a significant difference. Initial distances to the target were significantly different between experimental designs (stimulus: 477.4 ± 84.9 mm; ablation A1: 414.9 ± 49.5 mm, ablation A2: 470.3 ± 44.6 mm, ANOVA $p < 0.001$), success however, was not related to target distance (ANOVA $p > 0.05$).

4. Discussion

Collectively, our results clearly demonstrate that *S. entomon* responds to a distantly located target, and has the ability to locate it within a reasonable amount of time. Moreover, the animals seem to employ particular search tactics (or strategies) other than relying on purely fortuitous encounters. The use of distance- and contact-chemoreceptive appendages is instrumental in this respect. However, it is also apparent that in comparison with e.g. a decapod crustacean, *S. entomon* shows a considerable lack of precision and efficiency in locating a target even at close range, challenging our understanding of foraging strategies based on odor tracking behaviors.

Animal movements

Animal search patterns in general comprise a wide spectrum from seemingly random to highly deterministic behaviors. Contrary to human intuition, several studies have shown that a certain degree of randomness in the search process does not necessarily imply a reduced success rate in finding a particular target (Hoffmann 1983a, b, Motyka et al. 1985). Dependent on the environment and the distribution and density of resources, the stochastic constituents can have an adaptive value as not all random searches are similar successful (Heinrich 1979, Cain 1985, Bartumeus et al. 2008). However, most searching behaviors comprise both, undirected and directed elements (Bell 1990, Bartumeus et al. 2008). Which element is used depends on the sensory potential of the animal and hence its perceptive range in relation to target distance. Directed search can be differentiated into two elementary orientation mechanisms, taxis and kinesis. Both are understood as an orientation mechanism with regard to a physical or chemical gradient (for further differentiation of the terms and a critical discussion of the topic see Kennedy 1978). **Taxis** is based on the determination of the gradient direction, i.e. the direction of the largest local decrease or increase of a stimulus, which ideally corresponds to the target direction and hence the animals' path (Bovet & Benhamou 1990, Doucet & Dunn 1990). However, steering movements will inevitably be subjected to random variations either due to spatio-temporal fluctuations in the signal (e.g. turbulences) or, according to the modality, due to a low precision of the directional information gained (vision is in most cases more precise than chemoreception), both decreasing efficiency. Even so, given an attractive target, turning angles will incorporate a consistent bias towards that target, eventually leading the animal to it. In contrast, **kinesis** (i.e. differential klinokinesis) does not involve a determination of a gradient direction, but is considered as an assessment of the variation of the gradient potential during the animal’s movement which then regulates the frequency and amount of turning, eventually leading the animal to the source (Fraenkel & Gunn 1961, Benhamou & Bovet 1992). Therefore, once an animal has reached a target, especially as it is the case in *S. entomon*, one cannot but wonder whether this is the result of fortuitous luck or whether this has been achieved by an actual orientation mechanism, and if the latter is the case, which one was employed. The answer is anything but obvious as even apparently target-oriented paths contain random components and, dependent on its "efficiency", might be mistaken as kinesis or mere randomness. Given enough time and not considering the issue of mortality (either by natural causes of by predation), even completely random movements will at some point lead an animal to a target. The first step is therefore to consider the fundamental sensory potential of the animal in question.

The olfactory system - a general approach

Morphological as well as behavioral data strongly suggest that in order to e.g. find food most crustaceans employ a tactic orientation mechanism by the means of optical, mechanical or chemical stimulation. The detection of chemical stimuli is achieved by a large array of chemoreceptive structures that can be located on virtually every part of the body but are mostly concentrated on the appendages including first and second antennae, mouthparts and dactyli of walking legs (Heinen 1980, Derby 1982, Derby & Steullet 2001, Garm et al. 2003, 2005, Hallberg & Skog 2011). It is well established that spatio-temporal sampling of chemo- and mechanosensory information *via* antenna 1 provides navigating decapod crustaceans with the needed positional information relative to an odor plume ("odor tracking"). Walking legs and mouthparts play a subordinate role in search path control but become increasingly involved the closer the animal is to the source of a stimulus (Devine & Atema 1982, Moore et al. 1991, but see also Weissburg & Zimmer-Faust 1994). Whereas structure and function of olfactory systems are well understood in Decapoda, we are only beginning to explore the field of chemoreception in peracarid crustaceans. Few studies addressed olfactory guided behavior and foraging in marine Peracarida (e.g. Pynnönen 1985, Premke et al. 2003, Ide et al. 2006) but without considering apparent differences in the structure of the olfactory systems of Peracarida *versus* Decapoda. The general morphology of aesthetascs in *S. entomon* strongly resembles those in for example crayfish as representatives of typical decapods aesthetascs (Tierney et al. 1986, Sandeman & Sandeman 1996). However, with about 40 to 60 sensilla per antennule (Pynnönen 1985, Kenning & Harzsch 2013), they are relatively few in number compared to what is known from decapods (several hundred and up to over one thousand, Beltz et al. 2003). Moreover, they are sparsely innervated by dendritic processes (Pynnönen 1985), both suggesting a lower discriminative resolution at the perceptual level.

Taking up the trail

Besides the arrestment of movements, i.e. stopping and scanning of the environment, a frequently observed behavior in decapod crustaceans indicating olfactory arousal is referred to as antennal flicking, fast down-strokes of the antennal flagellum (Koehl 2011). This behavioral equivalent to sniffing greatly enhances odor capture by overcoming constraints imposed by fluid mechanics and neurophysiology (Schmitt & Ache 1979, Pravin et al. 2012). Antennal flicking narrows the boundary layer, thus shortening the diffusion distance for odor molecules to travel between the ambient fluid and the aesthetasc (Goldman & Koehl 2001). The periodic flushing and replacement of the fluid volume captured by the aesthetascs also resets the sensitivity of receptor cells which otherwise would adapt rapidly to continuously exposed odors (Schmitt & Ache 1979, Waldrop 2012). Neither in Kenning et al. (2015) nor in the present study has antennal flicking been observed in *S. entomon*. However, the animal can be seen repeatedly combing their antennules and antennae using the gnathopods. This behavior has already been reported by Pynnönen (1985) and Kenning et al. (2015), but also by Motyka et al. (1985) for crayfish. It is thus unclear whether this serves as a similar antennules cleansing purpose as flicking. In any case, a simultaneous comparison of chemical signal intensity between the chemosensory receptors on both sides of the animal might provide directional information, i.e. which side of the body is closer to the stimulus.

The olfactory system - a functional perspective

According to Schmidt & Mellon (2011), aquatic decapods receive and process chemical information in two different channels. “Olfaction” describes chemoreception mediated by the unimodal olfactory sensilla on the first pair of antennae, the aesthetascs. The second channel is described as “distributed chemoreception”, and is mediated by bimodal chemo- and mechanosensory sensilla located on the whole body surface. The essence of “olfaction” is understood as a quality assessment, providing a detailed representation of the complex chemical environment without particular reference to the source. In contrast, “distributed chemoreception” is thought to form representations of only a few active compounds within a somatotopic context that can serve to localize the source. Thus, an animal that is deprived of "distributed chemoreception" and that has to purely rely on "olfaction" might show differential chemokinesis. However, ablation experiments in the Caribbean spiny lobster *Panulirus argus* and the crayfish *Procambarus clarkii* demonstrated a functional overlap of the different chemosensory pathways. In both species the non-aesthetasc chemo- and mechanoreceptors alone are sufficient to locate the source of an odor (Giri & Dunham 1999, Horner 2004). Yet, no information on the actual searching behavior in these cases is available. In consideration of the efficiency which e.g. crayfish display to track odors however, chemotaxis is likely. Unfortunately, there is a considerable gap of knowledge on the distribution, morphology and physiology of the bimodal sensilla in *S. entomon*.

Information in addition to odors - assessing the water flow

As odorant transport is profoundly influenced by the prevailing flow conditions, the assessment of flow itself is an important source of information for animals. The stimulation of mechanoreceptors is integrated in the detection process, often by initiating an upstream movement. In fact, odor triggered rheotaxis (or anemotaxis in the case of terrestrial crustaceans and insects) appears to be quite common (Bell 1990, Baker & Vickers 1997, Vickers 2000, Weissburg 2000). Although it is not entirely evident how strong the rheotactic component influences the animal's detection of a stimulus, especially in the case of ablated animals, we propose that *S. entomon* does integrate hydromechanical cues in the detection process. However, as only antenna one and two ablated specimen verifiably displayed this behavior, the question of which receptive structures are involved in the detection, i.e. mechanical or bimodal sensilla, or non-antennal receptors, has to remain unanswered. A plethora of studies attested various decapods an exceptional ability to follow odor plumes, and to localize the source by employing temporally and spatially highly effective search strategies (e.g. Moore et al. 1991, Weissburg & Zimmer-Faust 1993, 1994; see also Vickers 2000). Crayfish, lobsters and crabs for example track odors using the lateral concentration gradient of the plume edge. This chemosensory information provided by their antennae as well as dactyli, in combination with chemo- and mechanosensory stimulation of various multimodal receptors, is used to orient upstream (Zimmer-Faust et al. 1995, Jackson et al. 2007, Page et al. 2011). As an animal moving comparatively slow with regard to the ambient flow, the stone crab *Menippe* is thought to use a time-averaging mechanism in analyzing an odor plume which, like in larger gastropods or echinoderms, dampens the effects of turbulences (Weissburg 2000, 2011, Ferner & Weissburg 2005). How this animal is able to actually locate a target of interest however, is unknown. Moore et al. (1991) analyzed the chemical orientation of *Homarus americanus* and found that similar to the present study, animals exposed to an odor, significantly reduced their walking speed. In stark contrast to the present study, path analyses showed that the lobster always moved in a rather straight line towards a target, not considering smaller sideway excursions. Consequently, turning angles as well as the directional error were in the range of a few degrees, and although no concentration parameter was calculated, judging from the frequency distributions provided it can be assumed to be quite high. Hence, there is little doubt that lobster (and likely Decapoda in general) use taxis as elementary orientation mechanism. Outside Decapoda, knowledge on the foraging associated movement patterns of crustaceans is scarce. Actively hunting copepods, despite their size, demonstrate a remarkable ability to follow the wake (and probably the chemical trail within) that a female conspecific or potential prey item leaves behind (Doall et al. 1998, Weissburg et al. 1998, Yen et al. 1998). However, long distance chemoreception is not satisfactorily documented in these animals (but see also Lombard et al. 2013), instead mechano- as well as contact chemoreception were suggested as the basic detection (Kiorboe 2013 and references therein). Premke et al. (2003) analyzed the food tracking behavior in deep sea amphipods and found conclusive evidence for long range chemoreception in *Eurythenes gryllus* (Gammaridea). Krång & Baden (2004) studied the responses of various gammarid species to conspecifics using a Y-maze (olfactometer) and demonstrated that males are capable of discerning between receptive and non-receptive females. As impressive these results are, the data provided do not allow for conclusions on behaviors on smaller spatial and temporal scales. For various insect species, long distance chemoreception has been demonstrated, and the importance of currents on searching behaviors and odor triggered migrations has been studied thoroughly (see Bell & Cardé 1984, Vickers 2000 for extensive summaries on this topic). Three main principles are discussed by which insects locate upstream targets. First, the animals detects a chemical stimulus which inititates rheo/anemotactic movements. This plume-directed movement can be continued for some distance even when the contact to the odor plume is lost or the current falls below a detectable level (Cardé 1984). Second, the detection of the plume edges sets a corridor in which the animal meanders upstream. The third mechanism is thought of as an internal motor program evoking zig-zagging movements through the plume. The turns and counter-turns at the plume edge (Tobin 1981) are guided by the detection of the gradient, similar to what can be observed in Decapoda (Bell 1984, 1990). Flying insects often overshoot the boundary before counterturning is initiated. However, they still show a considerable precision in following a plume and locating its source. With regard to isopods, so far only a single study has been conducted using an aquatic representative. Using a Y-maze bioassay, Kenning et al. (2015) tested the response of *S. entomon* to a range of odors the animal is likely to encounter in its natural habitat. In experimental conditions in which decapod species localized food related odors with high fidelity, *S. entomon* performed with very low precision in locating the odor source. Some studies are available on homing, food search behavior, and the responses to various odors of terrestrial isopods. These studies indicate that although oniscid isopods in principle are able to perceive olfactory stimuli, searching behaviors seem to be guided mainly by mechano- and contact chemoreception via antenna 2 (Linsenmair 1979, 1985, Hoffmann 1983a, b, Hoese 1989, Hoese & Schneider 1990, Zimmer et al. 1996) and by the use of particular search patterns comprising looping and spiraling movements, not unlike the behavior displayed by *S. entomon*. It also has to be noted that the evolutionary size reduction of the first antenna in Oniscidae had a cascading effect on the functionality of associated sensilla as well as the underlying neuronal substrate (Ábrahám & Wolsky 1930, Schmalfuss 1998, Harzsch et al. 2011). The major sensory organ of oniscid isopods is therefore the second antenna.

The neuronal substrate of olfaction

It has been shown that the fundamental architecture of the central olfactory pathway is basically identical in Decapoda and *S. entomon* (Kenning & Harzsch 2013) although distinct and, in terms of olfactory performance, probably crucial differences exist. Moreover, the relative volume of the olfactory centers involved in e.g. crayfish (Sandeman et al. 1992, 1995) is at least twice as large as in *S. entomon*. The aesthetasc input is relayed to a conspicuously organized brain region, the deutocerebral chemosensory lobe. The lobes are subdivided into functional units, the olfactory glomeruli, the number and size of which are considered to be a proxy for the overall sensitivity of the olfactory system as they are crucial for the animals ability to discriminate odorants (Beltz et al. 2003, Schachtner et al. 2005, Schmidt & Mellon 2011, Strausfeld 2012, Loesel et al. 2013). Whereas *S. entomon* possesses ca. 80 spherical glomeruli with a diameter of about 90 µm (Kenning & Harzsch 2013), Beltz et al. (2003) counted up to 500 elongated glomeruli in different crayfish species, reaching a length of almost 200 µm. However, a low glomerular count does not necessarily imply a low olfactory potential but may rather reflect the level of diversity of the antennal afferents. Distributed chemoreception of the non-aesthetasc pathway (Horner, 2004; Schmidt & Mellon, 2011) terminates together with mechanoreceptor afferents in a second brain area termed the lateral antenna 1 neuropil that in addition to the deutocerebral chemosensory lobe mediates a variety of odor-related behaviors (Steullet et al. 2001, 2002). In many decapods with good odor tracking, this area has a conspicuously stratified organization. The patterning is thought of as a somatotopic representation of the chemo- and mechanoreceptors along the first antenna (Schmidt & Mellon, 2011) that allows for the extraction of directional information from odor filaments moving across the antennae. In *S. entomon,* this area is comparatively small, of inconspicuous structure and shows no indication of stratification (Kenning & Harzsch 2013). This lack of structural diversification suggests that the center plays, if any, a subordinate role in odor tracking behaviors. Of considerable sensory significance however, is the second antenna as demonstrated above. Morphological data also strongly suggests a chemosensory function of the associated brain area. In the desert isopod *Hemilepistus reaumuri* as well as in *S. entomon* this area shows a microglomerular organization which usually is associated with a chemoreceptive function (Harzsch et al. 2011, Strausfeld 2012, Kenning & Harzsch 2013). For *H. reaumuri* it is known that mechano- as well as chemosensory input is provided by a compound sensillum located at the tip the second antenna, the apical sensory cone (Mead et al. 1976, Seelinger 1977). The importance of the second antenna in terrestrial isopods (as discussed in Hoese & Schneider 1990) led several authors to hypothesize that due to the functional loss of the first antenna in terrestrial representatives, oniscid isopods may have evolved new means for the detection of chemical stimuli as discussed above (e.g. Harzsch et al. 2011). The fact of a comparable structure in *S. entomon* (Kenning & Harzsch 2013) and the apparent relevance of antenna two for food search behavior in this species suggest that the functional shift towards the second antenna preceded the colonization of land by isopods and was probably already present in their last common ancestor.

Conclusion

Extrapolation of laboratory data to the field environment is a difficult task. It has to be stressed that confining test animals to an experimental setup and thus forcing them to make translocational and/or directional choices can produce a considerable bias in their behavior. The results however, might allow for approximations and highlight underlying principles that apply irrespective of environment. In the light of what is known about the chemoreceptive morphology (external and internal) as well as the behavior of *S. entomon* (Pynnönen 1985, Kenning & Harzsch 2013, Kenning et al. 2015), it is not unambiguously evident whether using their chemosensory system in order to find food is part of their foraging strategy because their major chemoreceptive appendages are not absolutely indispensable for *successful* foraging. However, it also is clear that *S. entomon* is advised to a complex interaction of long distance- and contact-chemoreceptive as well as mechanoreceptive appendages for *efficient* foraging. Although (the purely statistical) orientation tests (see above) suggest chemotaxis as orientation mechanism which are superimposed on rheotactic movements, the efficiency of the animal can easily be mistaken as kinesis or mere randomness in several experiments. While these terms seemingly define a reaction or mechanism precisely, it also suggests a "single-solution" system, thus camouflaging the importance of additional information which the animals may well be able to use. Furthermore, one should not forget the fact that biological mechanisms for optimizing the chances of "finding" unknown located targets should not necessarily be the same as those allowing for the "detection" of such target. Thus, the process of locating food itself might be optimized by a mechanism other than a sophisticated olfactory system, which in physiological terms is costly to maintain. With regard to their natural habitat which provides sufficient resources to maintain population densities as high as 120 individuals per square meter (Haahtela 1990), making use of sophisticated olfactory systems might not be as vital as in nutrient poor areas such as the deep sea. Moreover, *S. entomon* sustains starvation periods of several weeks and feeds on virtually everything it encounters and can handle (from carrion, small clams and amphipods to conspecifics, Green 1957; Haahtela 1990, Leonardsson 1991). Thus, by patrolling its habitat without cease, finding food through fortuitous encounters might be an appropriate strategy. After all, what is optimal depends on type, distribution and density of resources.

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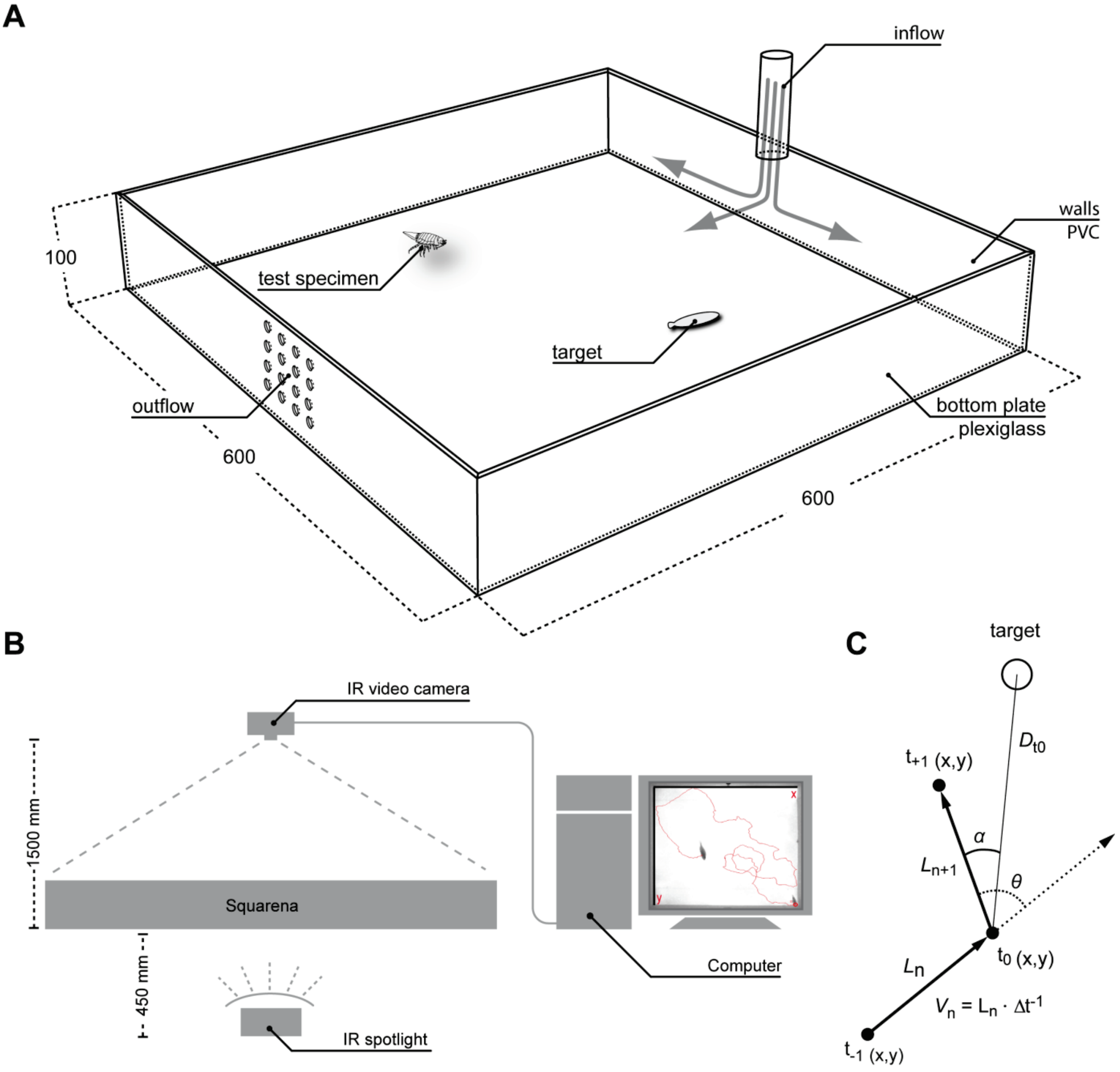


Fig. 1 | A,B "Squarena". Schematic views of bioassay - An infrared-translucent 3,600 cm² base plate with IR spot installed beneath and an infra red capable webcam connected to a PC enables real time observation and recording of the animals. Linear dimensions are provided in mm. **C Straight-line approximation of actual movement paths**. Calculation of movement parameters calculated from coordinates of successive locations $t_n(x,y)$: distance to the target (D_t), speed (V_n), corresponding step sizes (L_n), as well as turning angles (θ) and heading angles (α, directional error, the deviation between the current movement vector from a straight line towards the target)

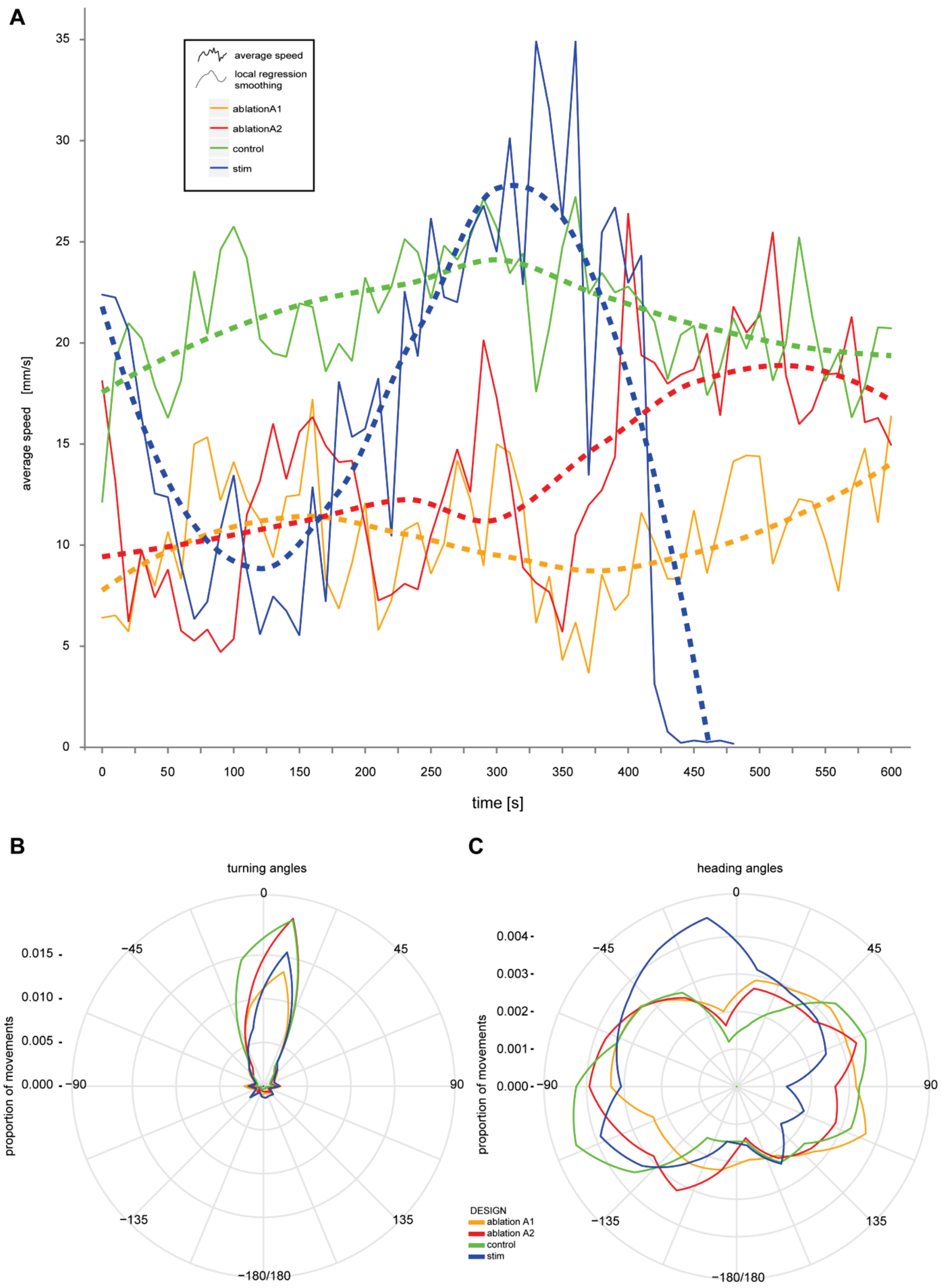


Fig. 2 | Mean speed, turning and heading angles - Comparison of basic movement parameter reveals striking differences between the four experimental conditions. **A** Speed of the animals over 600 seconds. Speed is given as a mean, averaged over 10 second intervals. The control group is characterized by a rather constant movement speed with only minor variation. Starting with a relatively high movement speed, stimulus treated animals display a sharp decline followed by an acceleration and a subsequent deceleration. **B** Mean turning angles of all experimental designs. All animals showed a distinct forward directed movement, with considerable differences in angular variation between experimental designs. **C** Mean heading angles for the experimental designs. Whereas the angles are bi- or multidirectionally distributed for the control and ablation of antenna 1 and 2, respectively, only stimulus treated animals showed a weak directedness towards the target

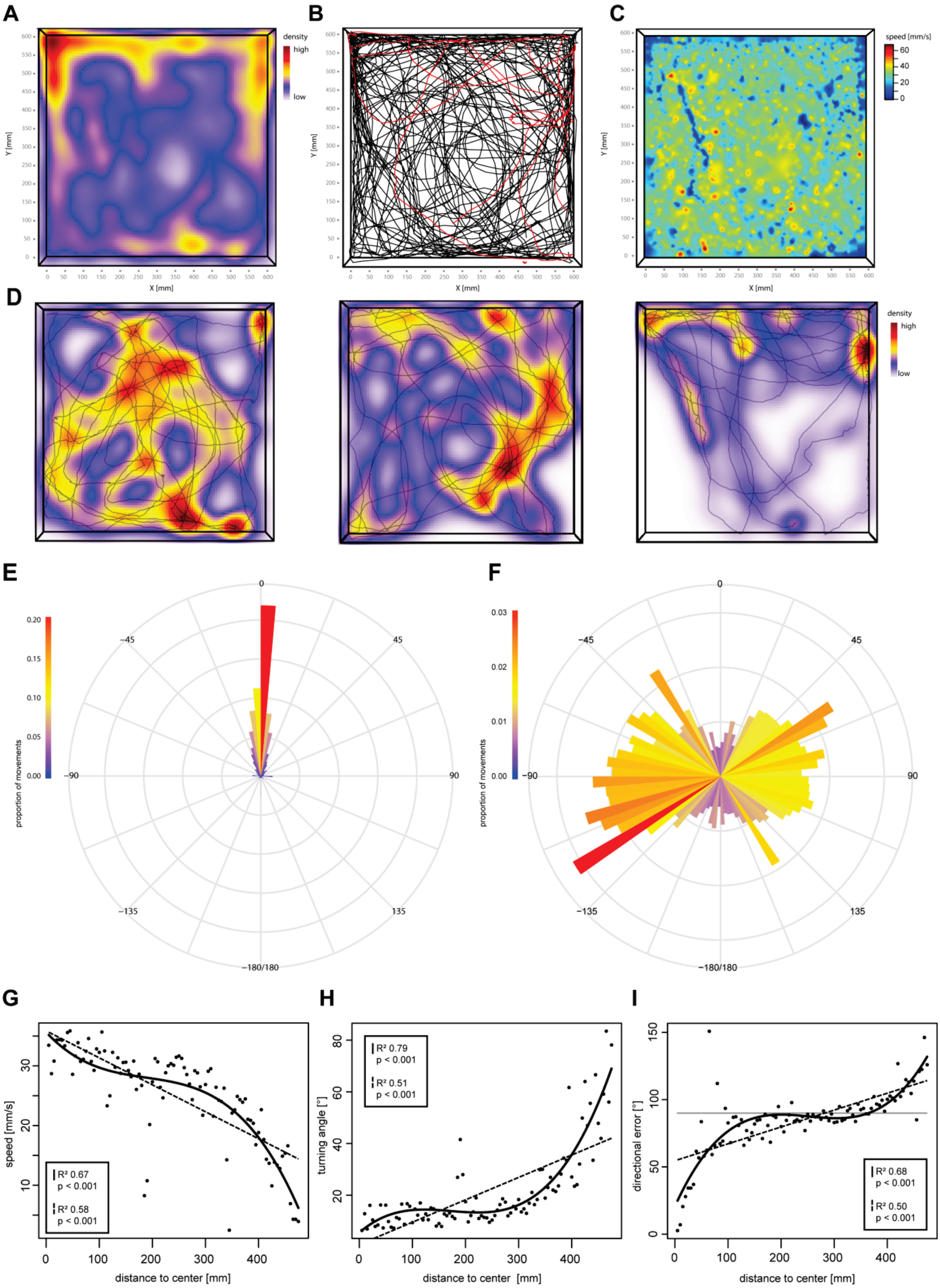


Fig. 3 | Negative control - A-D Top down view on the squarena, water inflow at the top **A** Density of occurrence of the animals (two trials excluded, marked red in B) **B** Visualization of "control" paths **C** Distribution of movement speed plotted over the squarena surface. With the exception of the squarena corners there is little spatial variation in movement speed **D** Three representative trials **E, F** Polar diagrams of relative turning and relative heading angles, both are given in degrees **G-I** Movement parameter as a function of target distance, provided are speed averaged over 10 seconds, absolute turning as well as absolute heading angles (all deviations from zero are given as positive values). Solid line: third order polynomial regression; dashed line: linear model fit. Determination coefficients as well as significance levels are provided

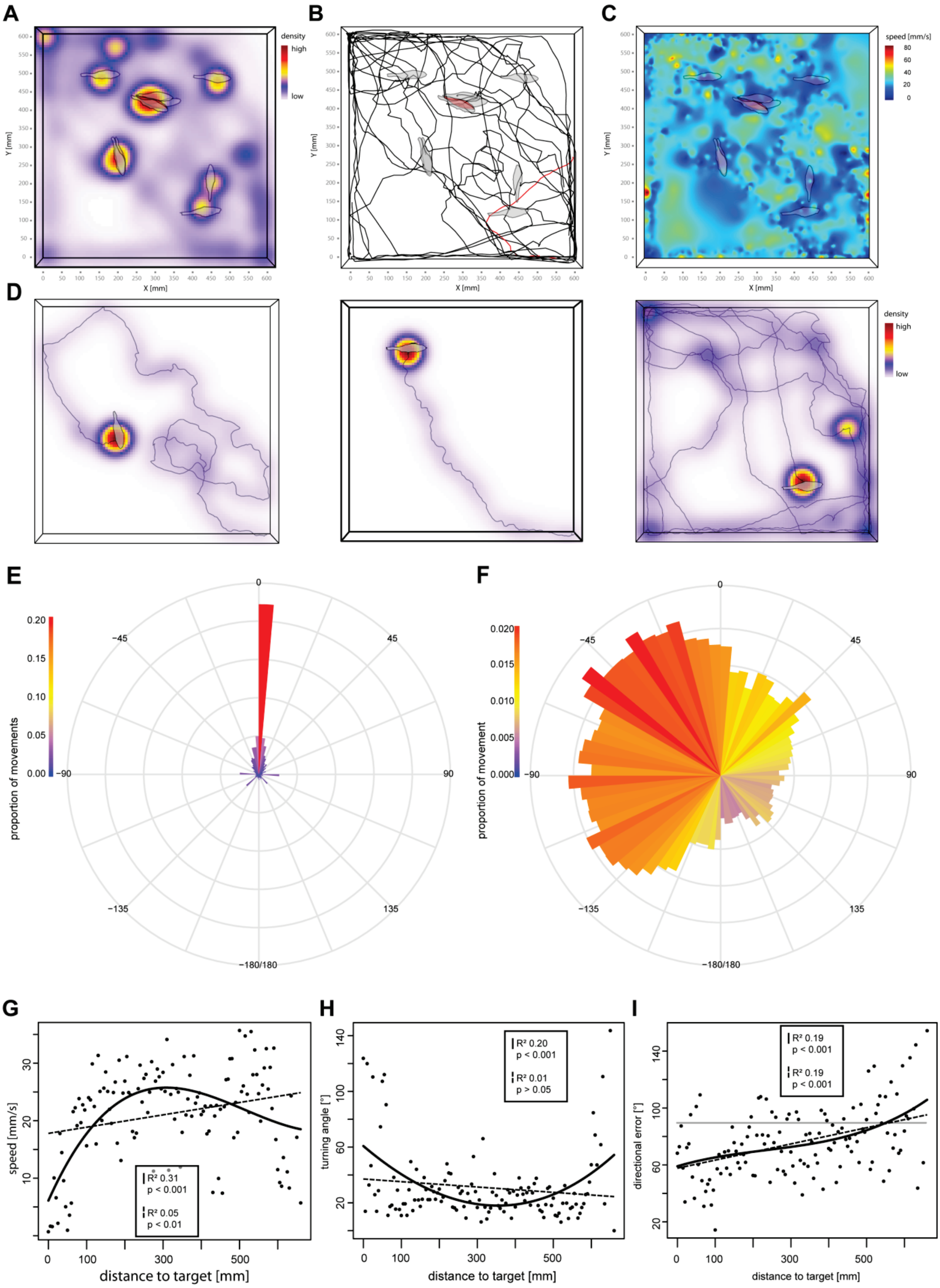


Fig. 4 | Stimulus treatment - A-D Top down view on the squarena, water inflow at the top **A** Density of occurrence of the animals (one trial excluded, marked red in B). Note that highest densities coincide with the location of stimuli **B** Visualization of "stimulus" paths. Note the abrupt directional changes of the paths **C** Distribution of movement speed. With few accelerations at the walls, it is consistently low over the whole squarena surface and even lower in the immediate vicinity of a stimulus. **D** Three representative trials demonstrating considerable variation in path structure **E, F** polar diagrams of relative turning and relative heading angles, both are given in degrees **G-I** Movement parameter as a function of target distance, provided are speed averaged over 10 seconds, absolute turning as well as absolute heading angles (all deviations from zero are given as positive values). Solid line: third order polynomial regression; dashed line: linear model fit. Determination coefficients as well as significance levels are provided

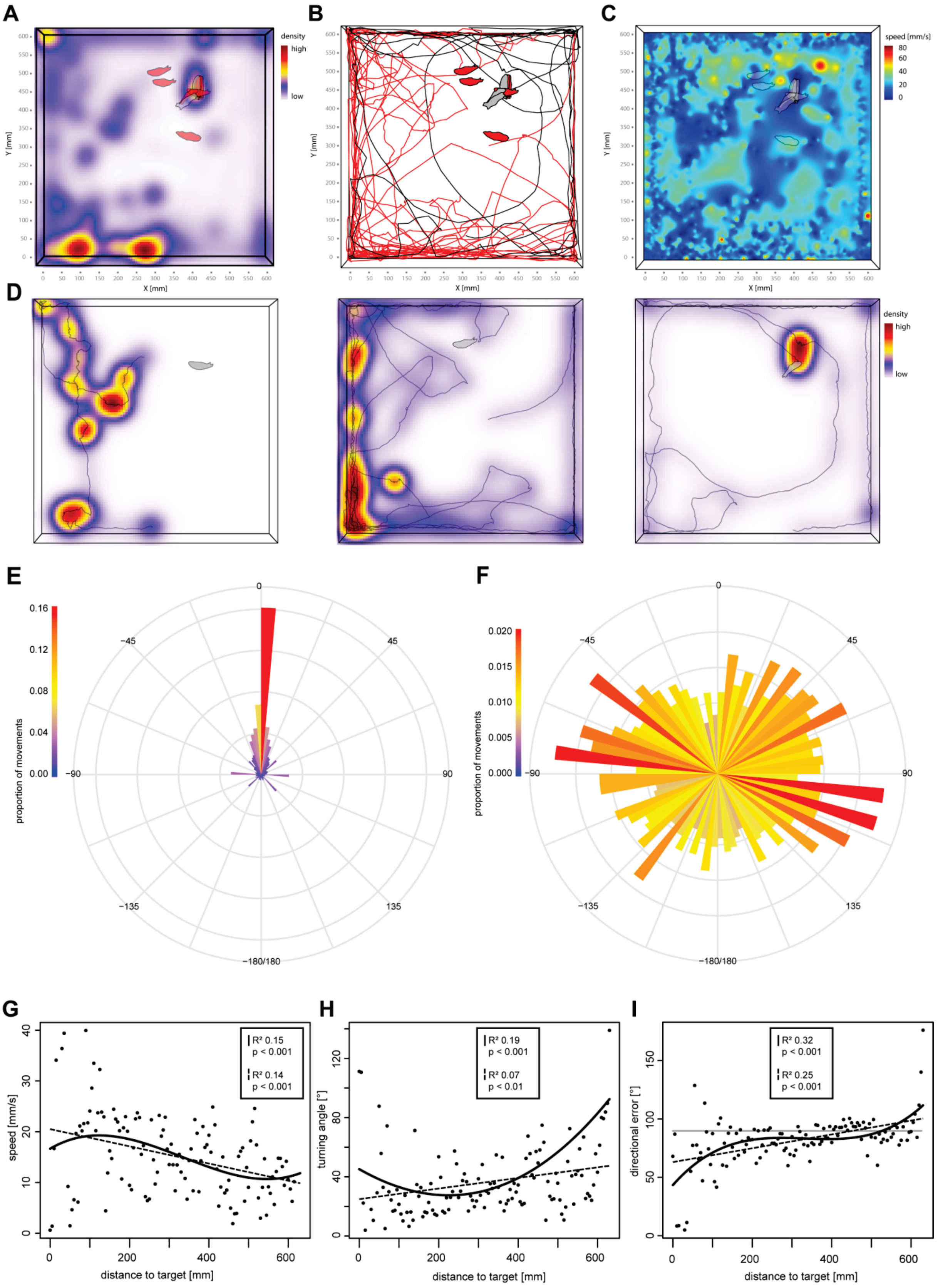


Fig. 5 | Ablation antenna 1 - A-D Top down view on the squarena, water inflow at the top **A** Density of occurrence of the animals. Note that highest densities do not coincide with the location of stimuli. Unsuccessful trials are colored red **B** Visualization of "ablation A1" paths **C** Distribution of movement speed plotted over the squarena surface. Note that the animals show a significant lower movement speed in comparison with control.

However, an acceleration in vicinity to the water inlet is also apparent. **D** Three representative trials demonstrating the variation in the animals path structure. Being in direct proximity to a target did not imply success in finding the target **E, F** Polar diagrams of relative turning and relative heading angles, both are given in degrees **G-I** Movement parameter as a function of target distance, provided are speed averaged over 10 seconds, absolute turning as well as absolute heading angles (all deviations from zero are given as positive values). Solid line: third order polynomial regression; dashed line: linear model fit. Determination coefficients as well as significance levels are provided

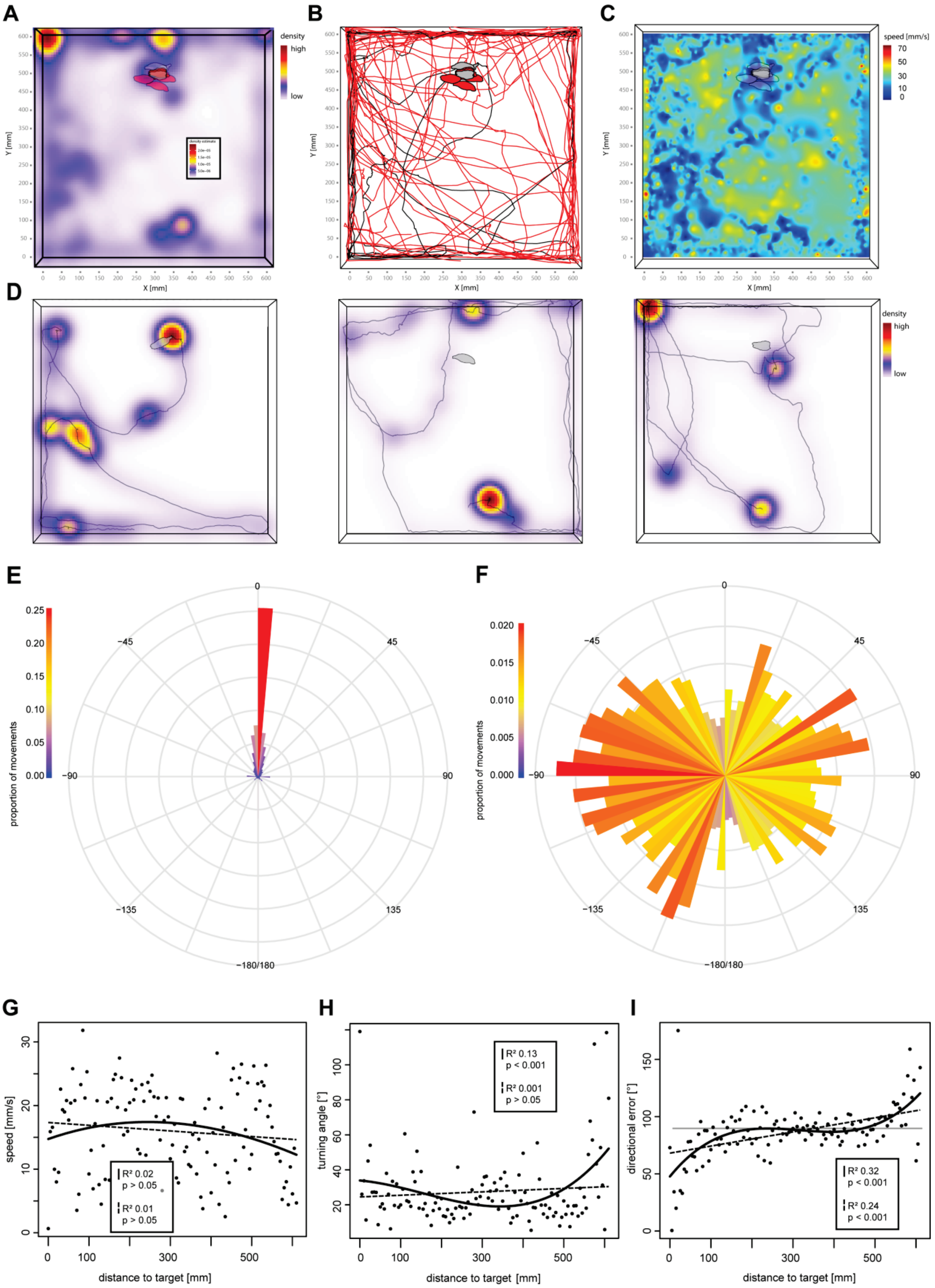


Fig. 6 | Ablation antenna 2 - A-D Top down view on the squarena, water inflow at the top **A** Density of occurrence of the animals. Note that highest densities do not coincide with the location of stimuli. Unsuccessful trials are colored red **B** Visualization of "ablation A2" paths **C** Distribution of movement speed plotted over the squarena surface. Note that the animals show a comparatively high movement speed in comparison with the stimulus treatment and ablation of antenna 1, resembling the control group. **D** Three representative trials demonstrating the variation in the animals path structure. Being in direct proximity to a target did not imply success in finding the target **E, F** Polar diagrams of relative turning and relative heading angles, both are given in degrees **G-I** Movement parameter as a function of target distance, provided are speed averaged over 10 seconds, absolute turning as well as absolute heading angles (all deviations from zero are given as positive values). Solid line: third order polynomial regression; dashed line: linear model fit. Determination coefficients as well as significance levels are provided

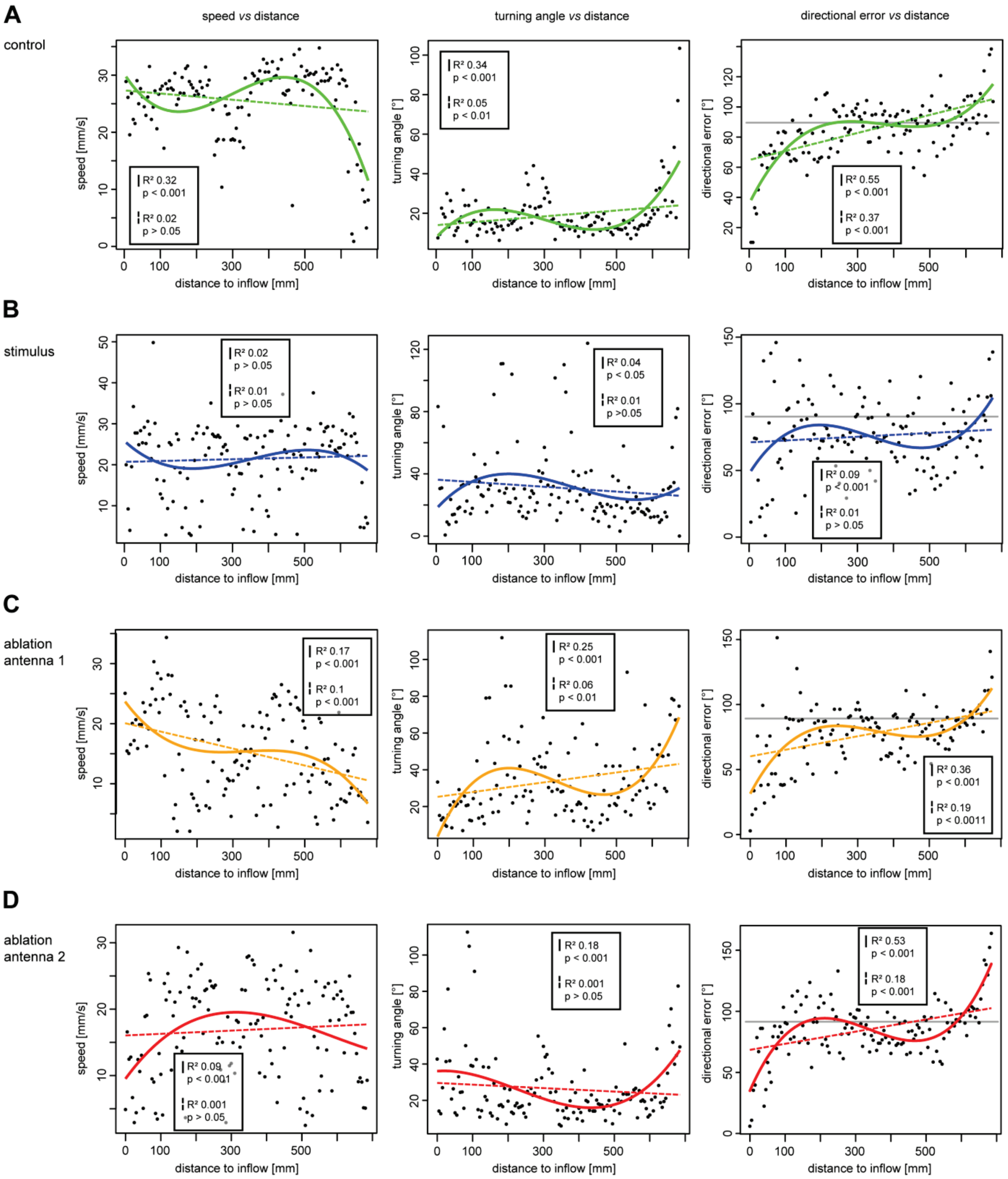
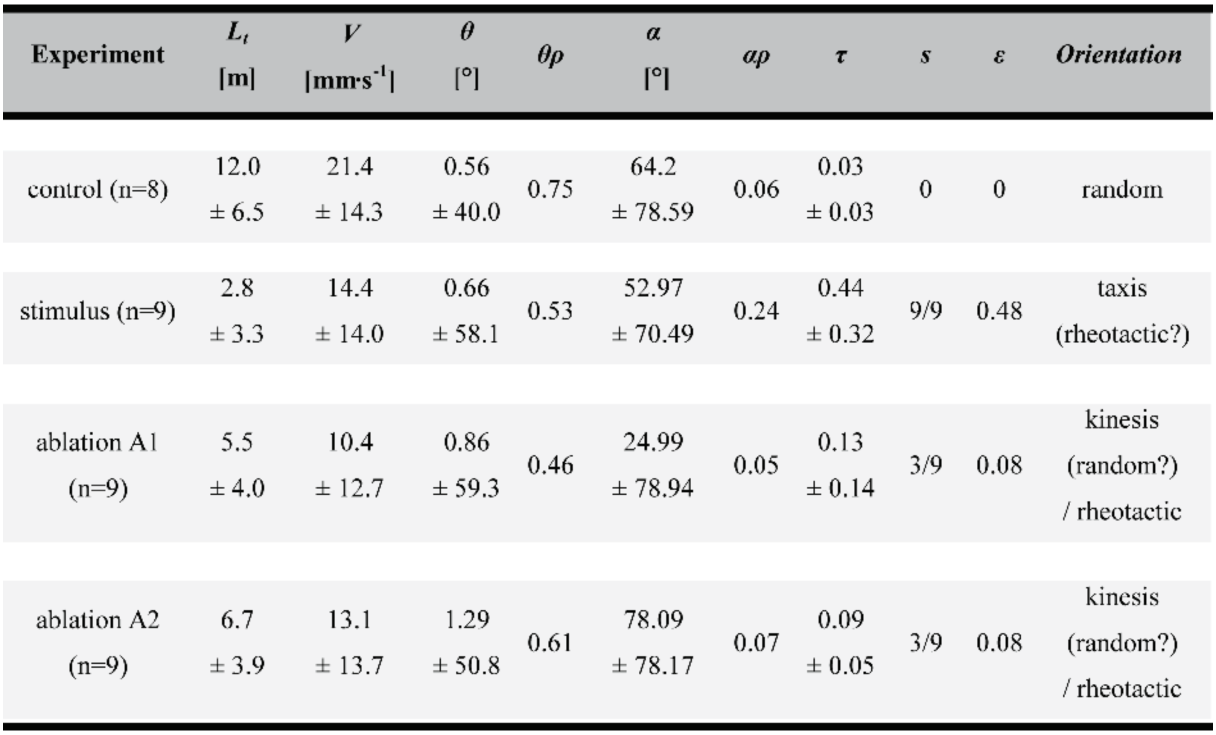


Fig. 7 | Movement parameter as a function of distance to water inflow - Provided are speed in mms $^{-1}$, absolute turning as well as absolute heading angles in degrees (all deviations from zero are given as positive values) with regard to distance to water inflow for **A** control, **B** stimulus experiments **C** ablation of antenna one and **D** antenna two. Solid line: third order polynomial regression; dashed line: linear model fit. Determination coefficients as well as significance levels are provided

Table 1. Mean Movement parameter L_t total distance, $\boldsymbol{V}$ speed, $\boldsymbol{\theta}$ turning angle, ρ concentration parameter, $\boldsymbol{\alpha}$ heading angle, $\boldsymbol{T}$ path tortuosity, $\boldsymbol{s}$ detection of stimulus, $\boldsymbol{\varepsilon}$ efficiency score and proposed **orientation** mechanism, ± standard/angular deviation.



Experiment	L_t [m]	V [mm·s^{-1}]	θ [°]	$\theta\rho$	α [°]	$\alpha\rho$	τ	s	ε	*Orientation*
control (n=8)	12.0 ± 6.5	21.4 ± 14.3	0.56 ± 40.0	0.75	64.2 ± 78.59	0.06	0.03 ± 0.03	0	0	random
stimulus (n=9)	2.8 ± 3.3	14.4 ± 14.0	0.66 ± 58.1	0.53	52.97 ± 70.49	0.24	0.44 ± 0.32	9/9	0.48	taxis (rheotactic?)
ablation A1 (n=9)	5.5 ± 4.0	10.4 ± 12.7	0.86 ± 59.3	0.46	24.99 ± 78.94	0.05	0.13 ± 0.14	3/9	0.08	kinesis (random?) / rheotactic
ablation A2 (n=9)	6.7 ± 3.9	13.1 ± 13.7	1.29 ± 50.8	0.61	78.09 ± 78.17	0.07	0.09 ± 0.05	3/9	0.08	kinesis (random?) / rheotactic

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Adaptive trends in malacostracan brain form and function related to behaviour.

2

ADAPTIVE TRENDS IN MALACOSTRACAN BRAIN FORM AND FUNCTION RELATED TO BEHAVIOR

David C. Sandeman, Matthes Kenning, and Steffen Harzsch

Abstract

In eumalacostracan brains, the sensory inputs from the various receptor systems distributed on the head appendages can be traced to their respective neuropils. The comparison of the brain "ground patterns" of a number of eumalacostracan species with that of a notional ancestral form is used here to explore the adaptive changes that have occurred during the evolution of the Eumalacostraca and that could be related to their adoption of particular habitats and lifestyles. We suggest that adaptations to habitat or lifestyle are mainly confined to receptor systems and within the organization of the primary sensory neuropils. Here the changes are often more quantitative than qualitative, although in some instances (e.g., the optic neuropils in stomatopods), neuropil structure can reflect the significant anatomical changes in the receptor organ. Eumalacostracan brains all contain neuropils that can be considered as "higher integrative centers" because they receive no direct inputs from primary sensory fibers and have no direct outputs to motor neurons. These centers are the hemiellipsoid bodies, the terminal medullae and the accessory lobes. At this level, both qualitative and quantitative differences between the species can be found, some of which have a phylogenetic basis (e.g., the appearance of the accessory lobes) while others are possibly related to behavioral adaptations associated with habitat or lifestyle (e.g., loss of the olfactory neuropils in desert isopods). The eumalacostracan brain therefore exhibits plasticity at both the quantitative and qualitative levels that matches the radiation of these animals across a wide range of habitats and the adoption of a variety of lifestyles.

INTRODUCTION

This chapter is concerned with the eumalacostracan crustacean brain, its ontogeny, and the relative differences in neuropil organization and size that may represent adaptive changes to the different habitats and lifestyles of different species.

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Animals reveal their presence either by their movement or by chemical cues. They live in a hierarchy in which they prey on those smaller or less able to defend themselves, or are preyed on by those larger or more ferocious than themselves. To avoid being preyed on, animals need to keep still and mask their own chemical cues. To survive, they need to detect the chemical cues or motion of potential prey. Significant areas of the brains of most animals are therefore given over to processing the inputs from the visual, chemical, and mechanical receptor systems. In the brains of the malacostracans, these are situated in the optic lobes and protocerebrum (vision, eyes), deutocerebrum (chemical and mechanical, antenna 1 or first antenna), and tritocerebrum (chemical and mechanical, antenna 2 or second antenna).

These functional divisions are blurred beyond the primary receptor projection areas by relays of second and higher order neurons. For example, responses to visual stimuli have been found in interneurons that project to deutocerebral neuropils (the accessory lobes in crayfish; Sandeman D. et al. 1995), and projections from the olfactory lobes are known to ascend to the lateral protocerebral neuropils (terminal medulla, Derby and Blaustein 1998; hemiellipsoid body, Mellon et al. 1992a, Wachowiak et al. 1996, Sullivan and Beltz 2001, 2005). Information concerning spatial orientation from statocysts is relayed to motor systems throughout the body (eyes, antennae, locomotory appendages, telson), and mechanoreceptive information from the tritocerebrum ascends to the deutocerebrum (Sandeman D. et al. 1995). The different modalities soon, therefore, become integrated into what is probably a diffuse neuronal "gestalt" spread throughout the brain. Nevertheless, it is still possible to distinguish between the *proportional* projections of the various sensory systems to different areas of the brain and draw some conclusions from this in terms of the ensuing behavioral patterns. Behavioral activity involves several levels of the central nervous system, and all except the simplest reflexes usually include the integrated input from many receptor systems. At the lowest level, actions require not much more than the basic framework of a coordinated motor system coupled to sensory feedbacks that regulate cyclic locomotory actions such as walking, running, hopping, swimming, and flying. Such feedback loops, coupled to specifically-tuned sensory systems, can rapidly compensate for unexpected external disturbance and prevent animals from stumbling on the ground or falling out of the air. At this level, the behavior is to a certain extent "automatic" and the associated neural components can be described and understood in terms of a relatively limited set of sensory and motor pathways. While such systems may appear self-contained, compensatory actions in freely-moving animals are integrated with higher order systems that determine the onset or cessation of bodily movement and control its direction and velocity. The basic motor framework also encompasses more than just locomotion, and there exist motor patterns that are responsible for grooming, feeding, fighting, mating, and so on, all of which employ the same appendages used in the compensatory reflexes and which are voluntarily regulated by the animal. Hence, the separation of behavior patterns into specific levels of complexity is less helpful than viewing the range of activities as a continuum extending from reflexive escape reactions, through compensatory actions to complex behaviors, which may indeed be dominated by one particular sensory input but seldom rely entirely on that modality. The complexity of the various behavior patterns could be defined in terms of the number of separate sequential components involved, their duration, and a requirement for learned elements. The neural substrate that is involved at this "cognitive" level in the behavioral continuum can be expected to be represented in the central nervous systems of all animals and not just those that are recognized as belonging to "higher" forms. "Cognitive" has been defined as "the mental action or process of acquiring knowledge through thought, experience and the senses" (*Oxford English Dictionary*), an ability seemingly reserved for the higher mammals alone. However, in some long-term crustacean behavior patterns (recognition of individual antagonists, social interactions, extensive seasonal migration, homing), learning and memory, both short and long term, appear to play a significant role, and even if these do not qualify as truly "cognitive," they come close to it.

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The literature on the nervous systems and behavior of the malacostracans is extensive and cannot be covered in a single chapter. We have therefore selected heavily, trusting that the examples we have chosen illustrate the structural plasticity found in the brains of malacostracans that accompanies their adaptive radiation within different habitats and their adoption of various lifestyles.

THE PHYLOGENY OF MALACOSTRACA

The crustaceans are a large and diverse group of arthropods. They range in size from tiny copepods of less than a millimeter in length, to long-legged spider crabs with a leg span of up to 1.8 meters. They are found in habitats extending from benthic volcanic vents, through coastal, estuarine, and freshwater habitats, to terrestrial areas including deserts. An extensive fossil record suggests that most major lineages of the crustaceans probably arose and diversified during the Precambrian. These then underwent a long period of independent evolution, leading to significant diversity within and among the groups. Genetic and developmental lability led to pedomorphosis, character convergence, and reversal, making the selection of useful phylogenetic characters difficult (Spears and Abele 1997).

While crustacean phylogeny is not the main issue in this chapter, a brief account of those groups that are included here and their relationship to the larger group that make up the Crustacea as a whole is relevant in spite of the difficulties mentioned above. We have relied on and slightly modified the phylogenies proposed by Scholtz and Richter (1995), Richter and Scholtz (2001), and Wirkner and Richter (2010).

Members of the Malacostraca are familiar to many and include the Stomatopoda (mantis shrimps), Decapoda (spiny lobsters, lobsters, crayfish, hermit crabs, crabs), Anaspidacea (Tasmanian mountain shrimps), Euphausiacea (krill), and Peracarida (shrimps, amphipods, isopods). For this account, we selected members of the Decapoda (Stenopodidea, Achelata, Homarida, Astacida, Anomala, Brachyura), Peracarida (Isopoda), and Stomatopoda, all of which are included in the Eumalacostraca (Fig. 2.1).

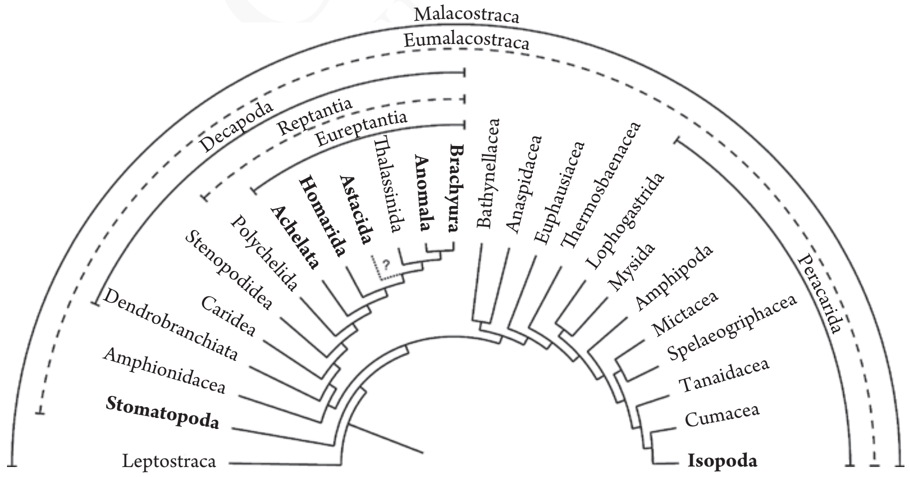

Fig. 2.1.
The phylogeny of the Malacostraca (based on Scholtz and Richter 1995, Richter and Scholtz 2001, Wirkner and Richter 2010). Bold text denotes the taxa from which examples in this chapter are taken. Question mark denotes uncertainty about this affiliation.

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THE MALACOSTRACAN BRAIN

Ground Patterns

An important feature of the Eumalacostraca is that their brains are "modular" and an anatomical segmentation is retained, allowing the sensory inputs from the various receptor systems distributed on the head appendages to be traced to their respective neuropils. The hypothetical "ground pattern" suggested for the decapods (Sandeman and Scholtz 1995) can be used as a template for other members of the Eumalacostraca (Stomatopoda, Syncarida, Euphausiacea, and Peracarida) because the same areas of neuropil can be identified in all of them. We use the terminology of Sandeman et al. (1992) with the exception of the optic ganglia. For these, we adopt the terminology of the homologous neuropils in insects, namely, lamina, medulla, lobula, and lobula plate.

We use the ground patterns here to compare the differences between the various species, which may be related to their adoption of particular lifestyles or habitats. We employ a color code (Fig. 2.2, center plate) to indicate four regions of the malacostracan brains (optic ganglia, protocerebrum, deutocerebrum, tritocerebrum) to which the different neuropils can be assigned based on their segmental ontogeny (see "Embryonic Development of the Brain") and identifiable subdivisions within these (Fig. 2.2, center plate).

In the ground pattern of a notional malacostracan, the brain is longer than it is broad (Fig. 2.2A) and includes a discernible lobula plate, and all lateral, medial, and midline protocerebral neuropils. The deutocerebrum lacks an accessory lobe and has only a single cluster of small "globuli" cells associated with each olfactory lobe. The lateral antenna 1 neuropils are as large as the olfactory lobes and are subdivided. The tritocerebral antenna 2 neuropils are elongated and relatively large. The generalized ground pattern of the Decapoda differs little from the malacostracan pattern except for the appearance among the Pleocyemata (Caridea, Stenopodidea, Reptantia) of a second cluster of "globuli" cells associated with each olfactory lobe (Fig. 2.2B). Figure 2.2 provides a general reference to the ground patterns of all the species considered in relation to their adoption of particular habitats and lifestyles.

Embryonic Development of the Brain

Clues to homologous structures, important for the derivation of ground patterns and phylogenetic relationships, can often be obtained from the study of the embryological development of the organisms of interest. Immunocytological techniques have extended the classical morphological studies and provided definitive descriptions of segmentation in crayfish (Fig. 2.3A,B) (Scholtz 1995, 1997, Alwes and Scholtz 2006, Sintoni et al. 2007, Vilpoux et al. 2008) and the maturation of neurons in the brain that express certain neurotransmitters (reviews Beltz 1999, Spindler et al. 2000) (Fig. 2.3C).

Developmental studies provide the following picture of brain segmentation in malacostracan crustaceans (Fig. 2.3D–F):

1. The ocular protocerebral region is associated with visual input from the median and compound eyes. The lateral protocerebrum is located in the eyestalks and comprises the terminal medulla and the hemiellipsoid body.
2. The median protocerebrum houses the central complex and the anterior and posterior median protocerebral neuropils.
3. The most proximal retinotopic neuropil, the lobula, derives ontogenetically from the terminal medulla and so is part of the lateral protocerebrum. The development of the lobula

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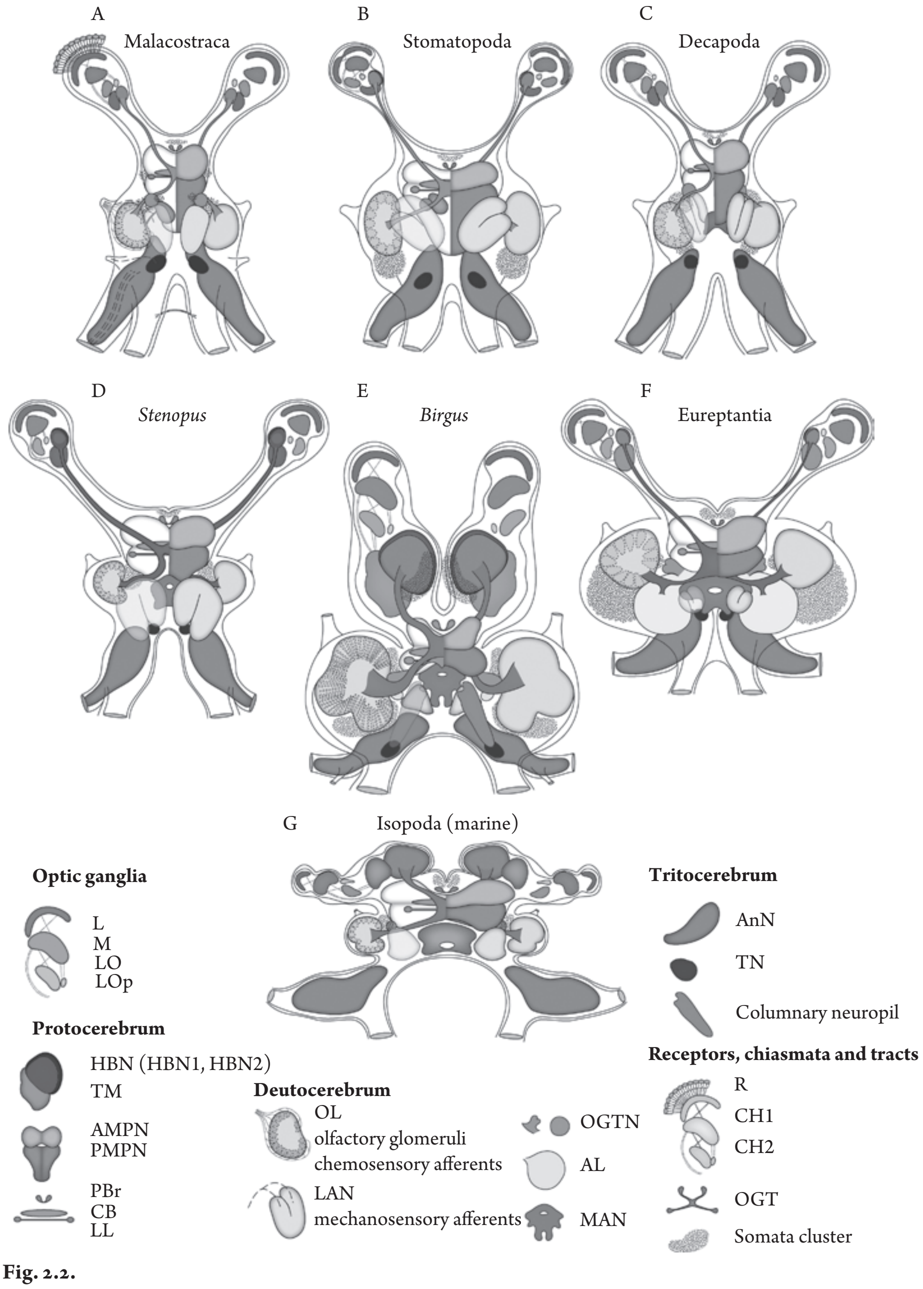

Fig. 2.2.
The ground and brain patterns of malacostracans. The subdivisions of the proto-, deuto-, and tritocerebrum can be identified by their shape and gray shade (**see color version of this figure in center plate**) provided in the key. (A) Malacostraca. (B) Stomatopoda. (C) Decapoda. (D) *Stenopus hispidus.* (E) *Birgus latro.* (F) Eureptantia. (G) Isopoda. Abbreviations: **Optic ganglia:** L, lamina; M, medulla; LO, lobula; LOp, lobula plate. **Protocerebrum:** HBN (HBN1, HBN2), hemiellipsoid body (subdivisions thereof); TM, terminal medulla; AMPN, anterior median protocerebral neuropil; PMPN, posterior median protocerebral neuropil; PBr, protocerebral bridge; CB, central body; LL, lateral lobe. **Deutocerebrum:** OL, olfactory lobe; LAN, lateral antenna 1 neuropil; OGTN, olfactory globular tract neuropil; AL, accessory lobe; MAN, median antenna 1 neuropil. **Tritocerebrum:** AnN, antenna 2 neuropil; TN, tegumentary neuropil; **Receptors, chiasmata, and tracts:** R, retina; CH1, first optic chiasm; CH2, second optic chiasm; OGT, olfactory globular tract.

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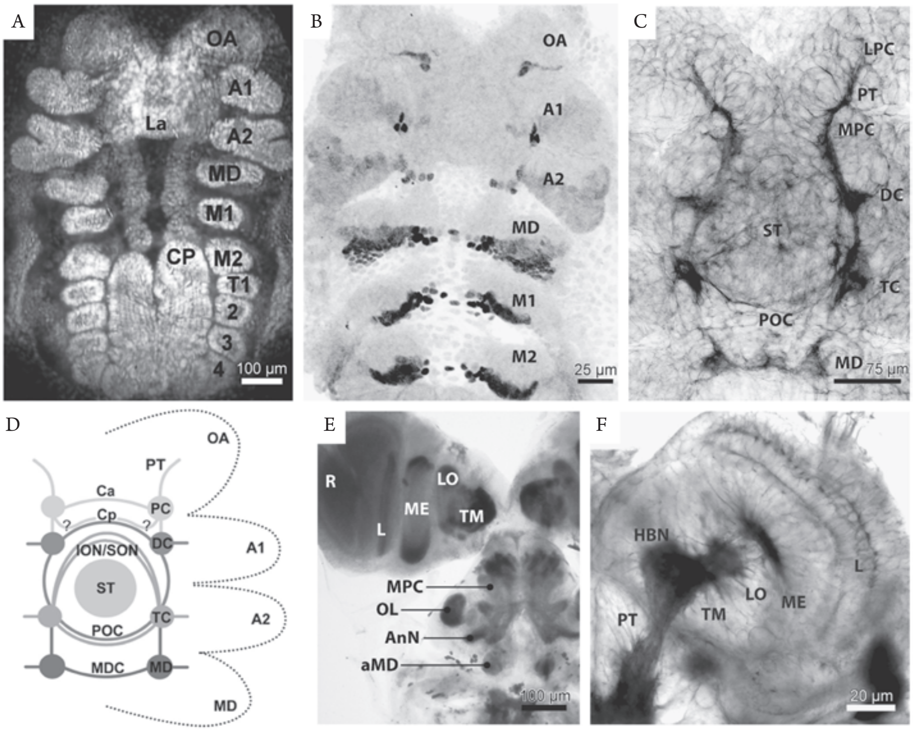

Fig. 2.3.
(A) Embryo of the marbled crayfish around 45% of embryonic development labeled with a general nuclear marker (from Seitz et al. 2005, with permission from John Wiley & Sons). (B) Engrailed immunoreactivity in an embryo of the marbled crayfish around 40% of embryonic development (from Sintoni et al. 2007, with permission from Springer) in which the labeled cells indicate the boundaries of the individual segments. (C) Developing brain in an embryo of the marbled crayfish slightly before 40% of development labeled with a probe against actin (from Vilpoux et al. 2006, with permission from Springer). (D) Schematic, idealized model of the egg-nauplius brain of crayfish, which, it is proposed here, conserves information on the possible appearance of the brain architecture of an early crustacean or even arthropod ancestor. The dotted lines depict the outlines of the egg-nauplius. A question mark denotes uncertainty about the connection. The four neuromeres of the naupliar brain are arranged in mirror symmetry across the horizontal center of the stomodeum (from Vilpoux et al. 2006, with permission from Springer). (E) Whole mount of lobster embryos *Homarus americanus* labeled against synaptic proteins (synapsin) to reveal the embryonic nervous system (Harzsch, Benton, Beltz unpublished, with permission from authors). (F) Development of the optic lobes in an embryo (E75%) of the crayfish *Cherax destructor* labeled with phalloidin, a probe against actin (from Vilpoux et al. 2006, with permission from Springer). Abbreviations: A1, 2, antenna 1 and 2 anlagen; aMD, anterior portion of the mandibular neuromere; AnN, antenna 2 neuropil; Ca, Cp, anterior and posterior pre-esophageal commissures; CP, caudal papilla; DC, anlage of deutocerebrum; HBN, hemiellipsoid body anlage; ION/SON, inferior and superior esophageal nerve; L, lamina; La, anlage of labrum; LO, lobula; LPC, lateral protocerebrum; M1, M2, anlagen of maxilla 1 and 2; MD, anlage of mandible and mandibular neuromere; MDC, mandibular commissure; ME, medulla; MPC, medial protocerebrum; OA, optic anlagen; OL, olfactory lobe; PC, protocerebrum; POC, post-esophageal commissure; PT, protocerebral tract; R, developing retina; ST, stomodeum; T1-4, anlagen of thoracic limbs 1 to 4; TC, anlage of tritocerebrum; TM, terminal medulla. Scale bars: A, 100 µm; B, 25 µm; C, 75 µm; E, 100 µm; F, 20 µm.

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plate is intimately linked to the lobula (Strausfeld 2005) and hence it too can be regarded to be part of the lateral protocerebrum. On the other hand, the first two retinotopic neuropils, the lamina and medulla, are protocerebral but seem to develop independently of the lateral protocerebrum.

4. Neuropils associated with the antenna 1 input such as the olfactory and accessory lobes and the medial and lateral antenna 1 neuropils are deutocerebral.
5. Neuropils associated with antenna 2 such as the antenna 2 neuropil are tritocerebral as is the tegumentary neuropil. The commissural ganglion has tritocerebral components as well as contributions from the mandibular neuromere. Traditionally, the esophagus has been thought to pierce the developing brain between the deuto- and tritocerebrum (Fig. 2.3C). However, recent developmental data revealing the presence of deutocerebral fibers *behind* the esophagus suggest that, as in pterygote insects, the esophagus actually pierces the deutocerebrum (Fig. 2.3D) (Vilpoux et al. 2006).

ADAPTIVE CHANGES RELATED TO BEHAVIOR

Complex behavioral patterns involve many receptor systems, but often one particular modality may dominate. Predatory animals, for example, frequently depend on their visual systems to detect and then pursue their quarries. This does not mean that the chemosensory or mechanoreceptive systems have no part to play, and quite often the different receptor systems will be employed in sequence during the behavioral procedure such as the use of tactile information during the actual grasping of the prey.

Habitat and lifestyle can play a significant role in determining the dominant receptor system. Predatory crustaceans, such as mantis shrimp, that live in conditions of high illumination may be expected to have capitalized on the visual system. Others, such as the fiddler crabs living in tidal mudflats whose dominant aspects of behavior (mating, feeding) take place in air, may have adopted visual systems that are particularly suited to the visual environment in which they live. Alternatively, if they are nocturnal or live in conditions of little or no light such as cave crayfish, they may have either very poorly developed eyes or none at all. Terrestrial crustaceans, such as land crabs, may have modified olfactory systems to operate in air or, like desert-living isopods, not have them at all and rely instead on contact chemoreception. Alternatively, animals that operate without good visual systems, or like spiny lobsters and Australian freshwater crayfish that are mainly active in conditions of poor light, or live in turbid water, can compensate with highly sensitive mechano-chemoreceptive appendages.

Examples that illustrate the above and are included here are grouped together under the subsections of visual, chemosensory, and exploratory mechano-chemosensory behavior.

Visual Behavior

Overview

Motion is perceived by an eye when an image moves across the photoreceptive cells in the retina. This can result from the movement of an object in front of the stationary eye or by the movement of the eye past a stationary object. As long as animals remain stationary, any motion in the visual field can be confidently assumed to be extrinsic to itself. During voluntary movements however, the situation is more complex because it is then necessary to distinguish between self-induced and extrinsic image movements. A strategy to confront this problem—development of eyes that can move independently of the body—has evolved several times. This allows an image to be

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stabilized, within limits, onto one area of the eye. Motion of small targets within this stabilized visual field is more easily detected than within an entire visual field that is also moving over the retina. A further development is found in active predators in which specific areas of the eyes, often with enhanced optical resolution, are fixated on an object of interest. In both cases, the strategy requires mounting the eyes in the head so that they can move in both vertical and horizontal planes, and the development of a neuromuscular system that can precisely control the eye movements in relation to the image on the retina.

Many species of crustaceans have stalked compound eyes, a highly-developed motor system that controls the eye movements, and a well-developed set of compensatory reflexes that are driven by the eyes themselves, the balance organs, and the proprioceptors in the legs. Amongst the malacostracans, stalked eyes are a characteristic feature of the stomatopods, decapods, all euphausids, and syncarids (except Bathynellacea, all of which are blind). The eyes of the peracarids however are sessile.

As examples of adaptations of visual systems related to behavior, we selected the stomatopods, fiddler crabs, and cave crayfish, and limited ourselves to comparisons of the anatomy of the optic neuropils (see also chapters 3, 8, 9, 18, and 19 in this volume).

Habitat and Lifestyle

Stomatopods

The four extant superfamilies of the Stomatopoda are the Squillidae, Lysiosquillidae, Gonodactylidae, and Bathysquillidae. All are marine, and members of the first three families live in water up to a depth of 300 m (Caldwell and Dingle 1975). The lysiosquilloid and gonodactyloid species predominate in brightly illuminated shallower water, and the squilloid species in the deeper areas. The bathysquilloids include the only species to extend their habitat beyond the 1,000 m mark (Marshall et al. 2007). Stomatopods are characterized by the development of the second maxillipeds as raptorial appendages that can be very rapidly extended to either crush (smashers) or impale (spearers) their prey. The gonodactyloids have adopted the smashing strategy and also tend to inhabit cavities in reefs from which they emerge to prey on reef organisms. Among the stomatopods, they occupy the greatest diversity of habitats. The squilloids and lysiosquilloids construct burrows in the soft substrata in which they live and have less armor than the gonodactyloid species (Caldwell and Dingle 1975). Lysiosquilloids lie at the entrance of their burrows with only the eyes and antenna 1 protruding through holes in a small mucus trapdoor over the entrance to the burrow. Small fish or other prey are “speared” as they pass by (Mead and Caldwell 2011).

The stomatopods exhibit complex behavior patterns in establishing their burrows or cavities, mating, and food capture. Those that live in shallower waters depend heavily on their visual systems, both for the recognition of conspecifics and prey items (Caldwell and Dingle 1975), but their olfactory and tactile senses are also important in some contexts. The eye movements of the stomatopods differ from the bilaterally-coupled motions of other stalk-eyed malacostracans; instead, each eye is able to move, chameleon-like, independently of the other and exhibits a high degree of freedom about all three rotational axes. They are almost continually in motion (Horridge 1978, Land et al. 1990) and will track rapidly moving targets (Cronin et al. 1988). The eye muscle systems that control these complex movements (Jones 1994) are unlike those known in the crabs (Burrows and Horridge 1968) or crayfish (Mellon 1977).

The eyes of the stomatopods are unusual among the malacostracans in that each eye is subdivided into three anatomically distinct regions in which a narrow midband of ommatidia separates two outer hemispheres. In line with the phylogeny of the stomatopods, the midband contains six rows of ommatidia in both the gonodactyloids and lysiosquilloids, two in the

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squilloids and none in the bathysquilloids. The midband contains 14 of the 16 different types of photoreceptors that are found in the eye (see Marshall et al. 2007 for review). The photoreceptor types in the hemispheres are like those seen in other malacostracans (Stowe 1980). Behavioral evidence indicates that at least the gonodactyloids and lysiosquilloids have both color and polarization sensitivity (Marshall et al. 1996, 1999). The tripartite retina of the stomatopods provides the optic ganglia with separate streams of information; the hemispheres, spatial and monocular stereoscopic information; the midband, color, ultraviolet light, and the plane of polarized light (Marshall et al. 2007).

Fiddler Crabs

Fiddler crabs inhabit tidal expanses of sand or mud and so live in a flat world. They dig burrows in the substratum, take refuge in these during high tides, and emerge at low tide to feed on the detritus that accumulates and remains in the surface layers of the mud or sand after the tide has receded. Some species live as mixed-age and -gender communities in relatively confined areas where an individual carries out its normal activities in a space that may not be more than a square meter. For the study of behavioral interactions of animals within a community, the fiddler crabs offer significant advantages and these have been exploited by many researchers (see Zeil and Hemmi 2006, and chapter 18 in this volume).

The lives of fiddler crabs are centered on their burrows, from which they make excursions. This exposes them to threats from predatory birds and from conspecifics that can gain access and possession of their burrows. Recognition of these threats and the response to them (i.e., immediate return to the burrow) depend entirely on the visual system, and an examination of their eyes reveals features that suit them perfectly for the role they play.

The eyestalks of the fiddler crabs are long and are folded laterally into grooves in the carapace when the animals enter their burrows. On emergence from the burrows, the eyestalks are extended vertically upward and are positioned close together over the midline of the body. The ommatidial surfaces of the eyes are located at the distal end of eyestalks and distributed around the surface of the cylindrical eyestalk. When extended, the two eyes together include a visual field of 360° in the horizontal plane. The corneal surface in the axial plane of the eyestalk, on the other hand, is almost straight. The vertical visual field is therefore a relatively narrow window that corresponds to an area just above and below the horizon. Many ommatidia are directed toward the horizon, which has the consequence of increasing the resolving power of the eye in this region. This is a useful feature when it is considered that it is in precisely this region of high contrast between the ground and the sky that objects larger than the animals themselves could appear and perhaps represent a predatory threat. Anything below the crab's horizon is either the same size or smaller than itself and so not a predatory threat, but possibly a conspecific rival (Zeil et al. 1986, 1989, see also chapter 18 in this volume).

Blind Crayfish

Several species of crayfish have invaded caves in recent geologic history and occupy many limestone cave systems in regions in North America. Among these are the obligate cave-dwelling (troglobitic) *Procambarus erythrops* (Mellon 1977) and *Orconectes australis packardi*, and the facultative cave species *Cambarus tenebrosus* (Cooper et al. 2001).

In caves or in aquaria in the laboratory, both *O. australis packardi* and *C. tenebrosus* sweep the long second antennae across the area in front of them while walking, implying a significant dependence on mechanical and chemical information. Recordings of the changes in heart rate indicate the sensitivity of cave crayfish to white light (but not to infrared or dim red light), waterborne vibrations, and chemical cues, although they may show no overt reaction to such stimuli

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(Li et al. 2000). The aversive reaction of *O. australis packardi* to a broadband light source (Li and Cooper 1999) may be mediated by photoreceptors situated in the caudal ganglion (Larimer 1966) or in the median protocerebrum of the brain (Page 1982, Sandeman et al. 1990).

Perhaps one of the most unexpected responses of both *P. erythrops* and *O. australis packardi* is their compensatory eye movements evoked by subjection to angular rotation about their long axes (roll) or when their bodies are held stationary while the substratum on which they are standing is rotated about the vertical axis (yaw). Recordings from motor nerves and eye muscles in *P. erythrops* and *Procambarus clarkii* (Mellon and Lnenicka 1980) show that these muscle systems are not only active but produce the correct compensatory response of the eyestalks. In sighted *P. clarkii*, these compensatory actions stabilize the image of the environment on the retina. The reflexes would appear redundant in the blind *P. erythrops* but are retained. The function is not clear. The protective reflex withdrawal of the eyestalk into its socket is also retained in *P. erythrops* (Mellon 1977).

Visual stimuli that evoke the behavioral "startle response" (raising the chelae into a defensive posture) in *P. clarkii* are ineffective in the other species, implying that they are unable to detect the change in light intensity that occurs when a hand is passed over the top of the animal, or that the stimulus has no meaning for the animals because small electrical responses can be recorded even from the cornea of *P. erythrops* following abrupt changes in light intensity (Mellon 1977) (see also chapters 8 and 9 in this volume).

When compared with the surface-living *P. clarkii*, the cornea and underlying retina of cave crayfish exhibit different degrees of reduction in the size of the eyestalk and retina and the presence of dark pigment behind the cornea. The eyestalk of *C. tenebrosus* is about half the size of *P. clarkii* but has a retinal surface that covers proportionately about the same surface area as that of *P. clarkii* (Cooper et al. 2001). The eyestalks of *P. erythrops* are cone-shaped with a much reduced retinal surface located at the apex of the cone. The cornea of *C. tenebrosus* is faceted, indicating the position of underlying ommatidia, whereas there are no facets on the cornea of *P. erythrops* (Mellon 1977). The eyes of *O. australis packardi* are the most reduced of the three species mentioned here, being mere stubs with no corneal surface or pigmentation (Cooper et al. 2001).

Central Neural Pathways and the Ground Pattern

The retinotopic projection pattern of photoreceptor axons of the ommatidia in the three different regions of the tripartite stomatopod eye is retained in the optic neuropils. Midband cartridge diameters are larger than those from the hemispheres and their projections are anatomically distinguishable as a separate area (accessory lobe) in the medulla and lobula (Kleinlogel et al. 2003). Although these central neural changes are related to the adaptation of the visual system for the complex predatory and social behaviors of the stomatopods, they are superimposed on the basic malacostracan ground pattern and appear to be confined solely to the optic ganglia (Fig. 2.2, compare A with B). The photoreceptor system on the other hand contains features that are unique to the stomatopods (Marshall et al. 2007).

While not as extensive as in the stomatopods, the adaptive changes in fiddler crabs are also predominantly confined to the receptor system and limited essentially to the elongation of the eyestalks, the particular distribution and size of the ommatidia over the retina, and the shape of the eye surfaces. The location, size, and gross appearance of the optic neuropils are little different from that of the basic ground pattern. Despite a complex social lifestyle, a uniquely adapted visual system, and a specialized habitat, the central nervous system ground pattern appears to have been conserved just as it has in the predatory mantis shrimp, which could hardly be more different in terms of habitat and lifestyle from the grazing fiddler crabs.

Blind crayfish provide an opportunity to deduce which neuropils are essential for vision and, because they have retained those in the lateral protocerebrum, also those important for chemo- or mechanosensory inputs.

The optic neuropils are reduced in size in both *P. erythrops* and *O. australis packardi,* and have lost the laminar organization that characterizes these neuropils in sighted crayfish (Mellon 1977, Cooper et al. 2001). While both species exhibit some sensitivity to light, they do not have a visual sense that includes the perception of motion or the formation of an image. However, despite this loss of a functional visual system, not only does *P. erythrops* retain an intact eye muscle system virtually identical to that of *P. clarkii,* but also the motor neurons that drive these muscles respond to nonvisual inputs that evoke oculomotor reflexes (Mellon 1977). The loss of the visual input has not extended to the motor system although the eye movements that are produced, and which in a seeing animal would serve to stabilize the visual image on the retina, are clearly redundant.

Strong connections via the large, fine-fibered olfactory globular tract that extends from the olfactory and accessory lobes (when present) to the hemiellipsoid body and to the terminal medulla are a common feature of the malacostracan ground plan. Despite the significant reduction of the optic neuropils in *O. australis packardi*, the numbers of axons in the projection of the olfactory globular tract to the eyestalk in these animals is greater than that of both the sighted *P. clarkii* and *C. tenebrosus* (Cooper et al. 2001). Here, it would seem that olfaction has become more relevant for the blind animals, and the hemiellipsoid body and terminal medulla are retained. The remainder of the brains of these animals has not been carefully investigated but most likely show little deviation from the eureptantian ground pattern.

Chemosensory Behavior

Overview

Chemoreception is perhaps the most important and most widely exploited sensory modality in the malacostracans, and even those animals with exceptional visual systems, the stomatopods, have highly-developed chemosensory receptor systems. A wide range of behaviors depend on the reception of chemical stimuli, and our current knowledge on this subject has been comprehensively reviewed in the multi-author volume *Chemical Communication in Crustaceans* edited by Breithaupt and Thiel (2011). Chemical stimuli are used in every aspect of the lives of crustaceans, and in many cases the behavior patterns are complex and of long duration involving a sequence of actions that depend on other senses (mechanoreceptive and visual) (Hay 2011).

Chemoreception in the malacostracans may be separated into two classes: "Olfaction" refers to the detection of odorants that are present in the surrounding medium. "Distributed chemoreception" refers to chemosensitive sensilla that are bimodal and respond to both chemical and mechanical stimuli (Schmidt and Mellon 2011, see chapters 3 and 10 in this volume).

In terms of possible adaptive changes in the chemoreceptive systems of the malacostracans, we confine ourselves here to a special set of chemoreceptive sensilla, the aesthetascs and their olfactory receptor neurons (ORNs), that are located on the first antennae, and the area within the brain to which these receptors project, the olfactory lobes. Olfactory information, like that of other sensory inputs, is relayed beyond the primary sensory neuropils to higher order centers in the brains of the malacostracans, and these projections are considered elsewhere in this chapter. As examples, we selected freshwater crayfish, coconut crabs, and woodlice (see also chapters 3 and 10 in this volume).

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Habitat and Lifestyle

Crayfish

Freshwater crayfish are found worldwide in ponds and streams and have been favorite experimental animals for neurobiologists for many decades. While the crayfish olfactory system clearly plays a part in finding nourishment, it plays a central role in mating behavior, including the associated establishment and recognition of dominance hierarchies.

The chemicals involved in mating and dominance contests are contained in the urine released from sphincter-controlled pores (Bushmann and Atema 1996) on the basal segment of the second antennae, and directed in water currents toward the conspecific receiver. These currents are generated by movements of the scaphognathite (gill bailer), maxillipeds, and fan organs associated with the mouthparts (Breithaupt 2001). The generation of water currents to disperse chemicals is critical in those species that live in stagnant water where diffusion is very slow. Both reproductively active male and receptive females of *Orconectes rusticus* and *Procambarus leniusculus* release pheromones during pairing (Berry and Breithaupt 2010).

Agonistic behavior in freshwater crayfish and the establishment of dominance has been comprehensively reviewed by Moore (2007) and by Herberholz in chapter 17 of this volume. Such contests between males begin with ritualized, non-damaging behaviors but can escalate to levels in which participants lose appendages. A characteristic of the behavior is the excretion by both contestants of pheromone-bearing urine. If the same animals are matched repeatedly, the fight durations will decrease and a stable dominance hierarchy will be established. The olfactory recognition of social status is a critical factor in the establishment and maintenance of the dominance hierarchy (see Breithaupt 2011 for review).

In the malacostracans, the detection of soluble or volatile odorants in solution or in air and at a distance from the source is carried out by the specialized olfactory sensilla on the first antennae, the aesthetascs (Hallberg et al. 1992, Hallberg and Skog 2011), and also by bimodal chemo-mechanoreceptive sensilla (e.g., hooded sensilla: Cate and Derby 2002), which are also responsive to chemicals that arrive in solution. The sensilla are probable homologs of those found broadly distributed on both the first antennae and body surfaces of the Palinuridae, Scyllaridae, and Nephropidae (Cate and Derby 2002, see also chapters 3 and 10 in this volume).

The structure of the aesthetascs in several species of freshwater crayfish is known (Tierney et al. 1986, Sandeman and Sandeman 1996) and conforms to the general malacostracan pattern (Hallberg et al. 1992, Hallberg and Skog 2011) in that they have a relatively thin-walled cuticle and up to several hundred chemosensory cells surrounded by enveloping cells. Each sensory cell has dendrites from which transformed cilia arise. In addition, the cuticle of the aesthetascs in spiny lobsters may act as a molecular sieve, selecting for molecules having a molecular weight of less than 8.5 kDa (Derby et al. 1997).

Coconut Crabs

Although the large coconut crabs, *Birgus latro*, of the remote Indian and Pacific ocean islands have long amazed biologists by their size and terrestrial adaptation, the details of their behavior and in particular their emphasis on aerial olfaction has only recently received focused attention (see Drew et al. 2010 for review), and much is still unknown. Nevertheless as a terrestrially adapted species, they are evidently opportunistic hunters and scavengers that are normally solitary but can be found in aggregations at sites that contain an abundant supply of a favored food source.

Copulation, during which a spermatophore is transferred to the female and deposited close to her gonopores (she has no seminal receptacle), takes place out of water as does the extrusion and fertilization of the eggs by the female (Drew et al. 2010). The egg mass is held beneath the

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abdomen of the female until shortly before hatching. At this time, the egg-bearing females move from their shoreline shelters out onto areas (intertidal flats or rocky ledges) that are washed by waves. Here the females cling to the substratum and release their eggs as the waves wash over them.

Aerial olfaction is clearly important for these animals. They are able to detect and find sources of odor and are particularly sensitive to oligosulfide compounds that are emitted from decaying meat (Stensmyr et al. 2005, Krieger et al. 2010, Hansson et al. 2011). The use of pheromones in intraspecific communication does not appear to have been studied, although it may well be used by males to find females during the mating period. Initial tracking studies point to the existence of a relatively restricted home range (600 m) in some animals, although individuals can undertake longer sorties (1600 m) (Hansson et al. unpublished data cited in Drew et al. 2010).

Even with the little information that we have on *B. latro*, here is a long-lived organism (estimated life span up to 40 years, Fletcher as cited in Drew et al. 2010) that exhibits a refined and olfactorily-oriented feeding behavior and a migratory-based mating procedure that depends on a guided and goal-oriented performance. The volume of the brain areas that are related to these behaviors is large in comparison with species with simpler lifestyles and more restricted habitats (Krieger et al. 2010).

The thin-walled aesthetascs of marine malacostracans are unsuited to terrestrial conditions because they would most likely dry out after prolonged exposure to air. Nevertheless, the coconut crab *B. latro* has retained both the aesthetasc sensilla and the basic olfactory system of the aquatic malacostracans although with some significant modifications. At the receptor level, the aesthetasc sensilla are present along the terminal portion of the lateral flagellum of the first antenna. They are short and scale-like and packed in rows along the ventral surface (which is directed forward in an active animal). The individual aesthetasc sensilla are also asymmetrical: the cuticle covering the exposed surface is thin, that of the unexposed surface, much thicker (Stensmyr et al. 2005, Hansson et al. 2011). Differences in diffusion coefficients of volatiles in air and chemicals in solution can also play a part in the design of aesthetasc sensilla in aquatic or land-living Crustacea (Mellon and Reidenbach 2011).

Modifications within the aesthetasc sensilla, such as the enclosure of the basal bodies and ciliary segments within a lymph space deep within the flagellum, are features that represent a convergence with the insects (Stensmyr et al. 2005). That the aesthetascs of the coconut crabs are sensitive to volatile odors and particularly to those that are also attractive to insects that feed on carrion has been convincingly demonstrated with behavioral and physiological techniques (Stensmyr et al. 2005, Hansson et al. 2011).

Wood Lice

The isopods occupy a wide range of habitats from marine (*Idotea baltica*), littoral (*Ligia oceanica*), terrestrial (*Porcellio scaber, Armadillium vulgare*), and desert (*Hemilepistus reaumuri*) (Harzsch et al. 2011). In terms of the degree of terrestrialness, isopods have achieved the highest level, defined as "fully terrestrial and able to conduct all biological activities on land." Here they surpass even the land crabs, whose early larval stages require a marine environment. The desert-living *H. reaumuri* is monogamous, lives in burrows, and has a remarkable kin recognition behavior based on specific chemical information, or badges. Family members are recognized according to the nature of a unique, non-volatile, chemical badge produced by the individual and combined with that of the family members and contained in the cuticle. When these animals molt, the badge is temporarily lost but it is regained by remaining in close contact with the family members within the burrows (Linsenmaier 1987, 2007; see Thiel 2011 for review).

The isopods provide a good example of the changes that can take place during the transition from marine to terrestrial habitats; the marine species such as *I. baltica* possess aesthetasc

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sensilla, albeit fewer in number than in the decapods (Harzsch et al. 2011). However, unlike the coconut crabs, the terrestrial isopods *P. scaber, A. vulgare*, and *H. reaumuri* have dispensed with the malacostracan olfactory system. Their first antennae are much reduced and the second antennae appear to have assumed dominance as sensory structures (Harzsch et al. 2011). In particular, and of relevance for the kin-recognition in *H. reaumuri*, is the presence of an apical sensory cone at the tip of the second antenna that is used to touch the cuticle of conspecifics and so identify the chemical signature signaling family or stranger (Linsenmaier 1987, 2007). The lack of aesthetasc sensilla does not, however, prevent some terrestrial isopods from detecting volatile, airborne odors (Thiel 2011).

Central Neural Pathways and the Ground Pattern

The aesthetasc sensilla on the first antennae in all the malacostracans are characterized by their exclusive and unilateral projections to the olfactory lobes, which are paired, usually spherical neuropils in the lateral deutocerebrum. The lobe contains cone-shaped columns of synaptic regions that radiate out from the center of the lobe and that are the target of the primary sensory axons of the aesthetasc sensilla (Sandeman and Denburg 1976). The regions between the columns, or glomeruli, are generally devoid of synapses (Sandeman and Luff 1973, Blaustein et al. 1988, Sandeman et al. 1992, Mellon and Alones 1993, Schmidt and Ache 1997; reviews: Schachtner et al. 2005, Schmidt and Mellon 2011, and chapter 10 in this volume). The total volume of the olfactory lobe, the volume occupied by the glomeruli, their number, and the number of aesthetasc sensilla that project to the glomeruli were all found to vary in a study of 17 decapod species (Beltz et al. 2003). The data show a continuum across the groups from those that favor a high convergence of ORNs from many aesthetascs onto few glomeruli, to those where ORNs from fewer aesthetascs are shared among many glomeruli. While not diagnostic, it would seem that those species that have the lowest number of sensilla per glomerulus are often found in estuarine, intertidal habitats. Intermediate ratios are found in several freshwater crayfish and marine crabs, whereas the highest ratios are found in the large marine clawed and spiny lobsters. Hence in terms of habitat, and perhaps also lifestyle, there appears to be an interaction between the receptor input and the central neuropil to which the olfactory receptor neurons project (Fig. 2.4).

The olfactory lobes of the crayfish most closely resemble those found in the eureptantian ground pattern (Sandeman et al. 1992, Sandeman and Scholtz 1995) (Fig. 2.2F). The olfactory lobe of the coconut crab is significantly modified, not only in that it has three instead of two subdivisions of the lobe but also because the associated accessory lobe is greatly reduced and the protocerebral hemiellipsoid body expanded (Fig. 2.2E) (Krieger et al. 2010).

The olfactory lobe in the coconut crab is also very large, occupying about 40% of the total brain neuropil volume and has over 1,000 glomeruli. In terms of the number of interneurons in the olfactory pathway, the coconut crab surpasses the insects (Krieger et al. 2010). This may not, however, be a feature related to the adaptation of the system to the detection and analysis of airborne odors because the neuropil volume, glomerular number and volume, the aesthetasc count, and the ratio of the number of aesthetascs per glomeruli in *B. latro* are very similar to that of the marine *Panulirus interruptus* (Table 2.1). The olfactory glomeruli of *B. latro* and *Coenobita clypeatus* are sharply divided into cap, subcap, and base regions, and these coincide with a regionalization of the hemiellipsoid bodies into a cap, core 1, and core 2 (Harzsch and Hansson 2008, Krieger et al. 2010).

The isopods, like the cave crayfish, provide us with an example of the consequence at the central level of the loss of a specific receptor organ. Olfactory lobes can be identified in the brains of *I. baltica* by their spherical shape and glomerular neuropils (Fig. 2.2G), whereas these

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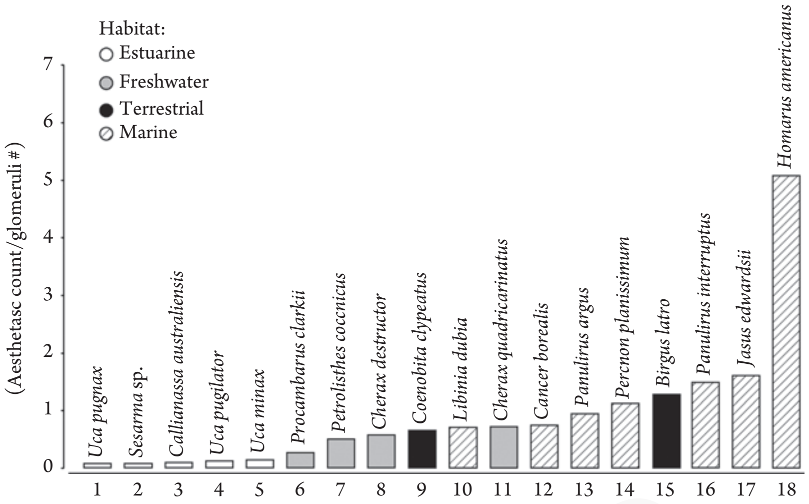

Fig. 2.4.
The ratio between the number of aesthetasc sensilla on the flagellum of antenna 1 and the number of glomeruli in the olfactory lobe reveals a tendency for the species to cluster into groups related to habitat: Species 1 to 5, estuarine; 6 to 8 and 11, freshwater; 9 and 15, terrestrial; 10, 12 to 14, and 16 to 18, marine (based on data from Beltz et al. 2003 and Krieger et al. 2010).

are absent from the brains of the terrestrial *P. scaber, A. vulgare*, and *H. reaumuri* (Harzsch et al. 2011). These isopods do not appear to be limited in their behavior in any way, having adapted sensilla on the second antenna to serve as both contact and olfactory sensilla (Thiel 2011). Also, while the large olfactory lobes (but perhaps more likely, the expansion of the hemiellipsoid bodies) may provide the coconut crabs with a neural substrate for olfactory memory, *H. reaumuri* is clearly able to learn and distinguish the chemical badges of its kin with no olfactory lobe at all. In summary, as seen in the visual system, adaptations to habitat and lifestyle would appear to be predominantly reflected in the receptor organs, and alterations in these are accompanied by central modifications involving the expansion or loss of elements of the original ground pattern.

Exploratory Mechano-Chemosensory Behavior

Overview

Many malacostracan species are active in the dim light of evening or early morning or at night, or live in water that is often turbid. Crustaceans employ their first and second antennae, mouthparts, and legs in the directed exploration of their surroundings. Many of the sensilla on these appendages are sensitive to both mechanical and chemical stimuli and hence, despite the active nature of this behavior, it is not purely "tactile" in the sense of the active touch or haptic behavior found in vertebrates. Nevertheless, it is likely that crustaceans are able to discern the spatial and chemical nature of their immediate environment by such means.

The anterior mechanosensory organs of the spiny lobsters and some crayfish are primarily their second antennae, particularly in cave crayfish but also in some epigean species, such as the Australian freshwater crayfish *Cherax destructor*, that are nocturnally active and frequently found in muddy or turbid ponds and streams. In spiny lobsters, the second antennae also play a defensive role, being relatively stiff with the basal segments covered in strong sharp spines. In some malacostracan species, particularly those with a diurnal lifestyle and that depend on

Table 2.1 Interspecific morphometric differences. Comparative data relating neuropil and glomerular volumes, glomerular numbers, and the ratios of aesthetasc sensilla to glomerular number across a range of reptantian species (redrawn from Beltz et al. 2003, with permission from Wiley)

Taxon	**Species**	**ON neuropil volume μm³**	**Glomerular volume μm³**	**Glomeruli number**	**Aesthetasc count**	**Convergence ratio (aesthetasc count/ glomeruli #)**
Achelata	*Panulirus interruptus*	344,922,004	287,884	1,202	1,786	1.486
	Panulirus argus	154,068,687	117,862	1,332	1,255	0.942
	Jasus edwardsii	591,956,438	616,475	961	1,537	1.599
Homarida	*Homarus americanus*	141,159,589	591,583	249	1,262	5.068
Astacida	*Cherax destructor*	24,187,019	110,975	230	130	0.565
	Cherax quadricarinatus	24,735,814	74,298	334	237	0.710
	Procambarus clarkii	9,790,377	19,585	503	133	0.264
Thalassinida	*Callianassa australiensis*	6,588,788	28,041	235	22	0.094
Anomura	*Coenobita clypeatus*	120,352,292	153,833	799	519	0.650
	Birgus latro	374,681,700	280,103	1,338	1700	1.270
	Petrolisthes coccineus	12,359,013	18,947	655	328	0.501
Brachyura	*Cancer borealis*	165,730,818	229,666	733	540	0.737
	Libinia dubia	20,327,317	39,338	454	319	0.703
	Percnon planissimum	28,765,244	58,705	495	555	1.121
	Sesarma sp.	6,617,077	14,887	446	33	0.074
	Uca minax	4,558,497	17,779	284	39	0.137
	Uca pugilator	3,114,604	13,14	234	28	0.120
	Uca pugnax	3,012,080	8,034	374	26	0.070

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vision, such as the stomatopods and rock crabs that inhabit rocky shores, the second antennae are small. In the stomatopods, however, it is the *first* antennae that play an important mechano-chemosensory role in mating behavior and that are coupled with the olfactory sense in the detection of pheromones (Mead and Caldwell 2011). The cleaner shrimp *Stenopus hispidus* has very long first and second antennae. The examples we consider here are the Australian freshwater crayfish, gonodactyloid stomatopods, and a stenopid cleaner shrimp (see also chapter 3 in this volume).

Habitat and Lifestyle

Crayfish

Cherax destructor, an Australian freshwater crayfish, is nocturnally active and forages on plants, other arthropods, and carrion. It is solitary and lives in complex underwater burrows that it defends and can use to survive periods of drought (Olszewski 1980, Reynolds 1980). Individuals have a home range that they patrol nightly, at times leaving the water to forage on the banks (Reynolds 1980). Given the relatively complex underwater environment, and the establishment of a home burrow, it is more than likely that the animals have a good spatial knowledge of their near environment and that they use mechano-chemosensory information to find their way around in the dark and at times muddy waters.

Cherax destructor, if placed overnight with fish in an aquarium, will capture and eat these (Sandeman unpublished observation). Given that capture took place in low light conditions, one might assume mechano-chemosensory mediated behavior although some crustaceans and other arthropods have highly sensitive eyes that can operate in extremely low light intensities (see chapters 8 and 9 in this volume), so that vision cannot be totally ruled out in the above example. On the other hand, *Euastacus spinifer*, another Australian freshwater crayfish, actively hunts for tadpoles by antennating the substratum and not relying on vision (Turvey and Merrick 1997).

The visual system in *C. destructor* appears to play a less important part in the search for stationary food items; both sighted and blinded crayfish stimulated by food odor will immediately begin to search the area, antennating the area around and in front of them. Provided with a small novel object, such as a polystyrene ball, both sighted and blinded animals will attack and attempt to eat this but only if they come across it with antennae, chelae, or legs (Zeil et al. 1985).

Touching the second antennae of sightless *C. destructor* results in the animal lunging rapidly toward the point of contact and striking with its chelae. The direction and distance covered in such attacks are correlated with the angle of the antenna at the moment of contact and the point of contact along the antennal flagellum (Zeil et al. 1985, Sandeman and Varju 1988). Crayfish will also direct their second antennae at the source of a distant mechanical disturbance (Tautz et al. 1981, Masters et al. 1982, Tautz 1987).

Like many other animals, when *C. destructor* is placed in a novel environment, it will explore. In an aquarium containing objects, blindfolded animals walk along the walls and stop to explore any object they come across with the second antennae. Placed in an arena empty of objects, blindfolded animals walk around the walls, "trailing" the nearside antenna along the wall. The antenna is flexible and "rolls" partially back past the animal as it trails the flagellum along a surface (Sandeman 1989). After a series of four repeated exposures to an empty arena, the period of exploration decreases, signifying habituation. An immediate increase in the exploratory activity (i.e., dishabituation) follows the introduction of short partitions projecting from the side walls of the arena. That such dishabituation also follows the removal or repositioning of the partitions implies that the animals are able to detect changes in the topography of their environment using mechano-chemosensory inputs alone and retain this information for at least 24

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h. Animals with immobilized second antennae no longer respond to topographical changes in the arena (Basil and Sandeman 2000). Part of the mechano-chemosensory exploratory strategy of *C. destructor* apparently depends on a bilateral comparison between the information received by the two antennae because in a T maze, animals with one immobilized or denervated antenna turn more often toward the untreated antenna (McMahon et al. 2005).

The basal segment of the second antenna in crayfish is formed from the fusion of basipodite, ischiopodite, and meropodite. The flagellum of the second antenna is borne on the next proximal segment, the carpopodite, and is devoid of muscular tissue. The articulation of the basal segments is monitored by chordotonal organs. The same anatomical organization occurs in spiny lobsters (Rossi-Durand and Vedel 1982) and American lobsters (Sigvardt 1977).

In *C. destructor*, the articulation of the basal segments of the second antenna is such that the flagellum can be directed at any point in space within a solid angle in the horizontal plane from about 10° on the contralateral side to 170° on the ipsilateral side. The excursion in the vertical plane extends from 10° below the horizontal in front of the animal to almost 180° at the back (Sandeman 1985). The second antennae in *C. destructor* are almost as long as its body, a feature shared with spiny lobsters and troglobitic crayfish.

Mechanoreceptors on the second antennae of several species of crayfish have been described (*Astacus leptodactylus*: Tautz et al. 1981, Bender et al. 1984; *Procambarus* spp.: Taylor 1968, 1975a, 1975b) and are known to be highly sensitive to water vibration (Tautz et al. 1981, Masters et al. 1982). Four different types of setae were identified in scanning electron micrographs on the flagellum of the second antenna in *C. destructor.* These are the following:

1. Feathered procumbent setae that project across the arthrodial membrane linking the segments of the flagellum. These do not appear to be innervated in either *C. destructor* (Sandeman 1989) or in *A. leptodactylus* (Bender et al. 1984).
2. Short smooth setae that project at right angles to the flagellum and that comprise several subtypes. They resist deflection toward the base of the flagellum but hinge easily forward to lie flush against the flagellum.
3. Long smooth setae with their bases contained in large sockets.
4. Short stubby peglike setae (Sandeman 1989). Similar setal types with the exception of the peg setae occur on the second antennae of both *Homarus americanus* (Tazaki 1977) and *A. leptodactylus* (Tautz et al. 1981).

The long smooth setae in *C. destructor* are highly mobile and can be observed to move with the slightest disturbance of the bathing medium. Electrophysiological measurements showed that responses to the motion of the bathing medium are abolished if all the long smooth setae are plucked off (Sandeman 1989). The short smooth setae are relatively insensitive to water motion, but respond with rapid phasic bursts when deflected.

Stomatopods

In stark contrast to the crayfish, the second antennae of the stomatopods play a very different role. The flagellum (endopodite) of the second antenna is relatively short. The exopodite is modified into a large elongated oval-shaped scale that in some animals is highly colored and used in agonistic displays. Such displays involve raising the cephalothorax, extending the first antennae and the scales of the second antennae, and the spreading and extension of the raptorial meri (Caldwell and Dingle 1975).

Mating in these animals involves a complex process in which the males approach the females at the entrance to their burrows. Chemical signals are sent by both male and female individuals and driven toward each other with circular propulsive movements of the maxillipeds.

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The male also investigates the female using the first antennae (Mead and Caldwell 2011), possibly to detect both distant and contact pheromones with the aesthetascs and bimodal mechano-chemoreceptors.

The behavioral observations of the stomatopods would suggest that the first antennae have assumed the mechano-chemosensory function of the second antennae of the decapods and play an important role in both mating behavior (Mead and Caldwell 2011) and prey capture (Schaller 1953).

Cleaner Shrimps

Cleaner shrimps are found in the Caridea (*Urocaridella* spp., *Periclimenes* spp., *Lysmata* spp.) and in the Stenopodidea (*Stenopus* spp.). Although belonging to three separate subfamilies within these taxa, they share some significant common anatomical and behavioral features. All are colored and have both red and either white or yellow bands or spots on their bodies and legs, although in many species the rest of the body is transparent. All have long, flexible first and second antennae, and in *Lysmata amboiensis* and *Stenopus hispidus* the first antennae are particularly noticeable, the two rami being almost as long as the second antennae.

Organisms that live by cleaning others have to advertise their services in some way and avoid being taken as prey. Cleaner fish have a characteristically banded body coloration and undertake "dances" in which they swim up and down in the water column over their cleaning stations which are visited by much larger client fish (Grutter 2004). Cleaner shrimps also use this combination of repeated oscillatory motion coupled with color banding. Individuals of *Urocaridella* spp. signal their presence and readiness to clean by rocking their bodies back and forth. *Stenopus* spp. and *Periclimenes* spp. employ this strategy and in addition whip their antennae back and forth when potential clients are nearby (Becker et al. 2005).

The relatively long first and second antennae and the emphasis placed on the coloring and movement of these in the unique behavior of these animals would suggest some discernible adaptive changes within the brain. There do not appear to be any available data on the nature of the sensilla on either the first or second antennae of these animals.

Central Neural Pathways and Ground Patterns

In the malacostracan ground pattern, axons from the second antennae project to the antenna 2 neuropils in the tritocerebrum, and this is the pattern in the crayfish, stomatopods, and cleaner shrimp *S. hispidus* (Fig. 2.2D). Despite the apparent dependence of the crayfish *C. destructor* on the input from the second antennae, the antenna 2 neuropils are not particularly large. They do, however, exhibit a striated appearance in histological sections caused by fiber bundles that run across the longitudinal axis of the neuropil, although this is not as pronounced as that found in *Penaeus monodon* and *Pagurus bernhardus* with their very long second antennae (Sandeman et al. 1993, Krieger et al. 2012) or in *S. hispidus* (Sandeman unpublished observation). While not confirmed, it is conceivable that the striations within the neuropil represent an ordered spatial projection from mechanoreceptors along the length of the flagellum. Unlike the stomatopods and the cleaner shrimps, the crayfish have large accessory lobes that receive projections from the antenna 2 neuropils (Sandeman D. et al. 1995).

In the stomatopods, the *first* antennae have become dominant mechano-chemosensory organs, while not losing their olfactory role provided by the aesthetascs. The projection of the axons from the first antennae conforms to the malacostracan ground pattern: aesthetasc sensilla project exclusively to the olfactory lobes while all the other types end in the lateral antenna 1 neuropil in the medial deutocerebrum. As in other malacostracans, this is not a complete separation between chemoreception and mechanoreception because bimodal receptors that are

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both chemoreceptive and mechanoreceptive project to the lateral antenna 1 neuropil. The aesthetasc/olfactory lobe system mainly mediates complex behaviors associated with the detection of intraspecific chemicals such as recognition of dominance and kin and in mating behaviors (Schmidt and Mellon 2011, chapter 10 in this volume).

The lateral antenna 1 neuropil in the stomatopods is large and bilobed (Fig. 2.2C). The bilobed nature of the lateral antenna 1 neuropil is also a feature of the malacostracan ground pattern; however it is more pronounced in the stomatopods. In addition, the stomatopod lateral antenna 1 neuropils exhibit a pronounced pattern of fiber bundles running transversely to the long axis of the neuropils.

The elongated first and second antennae of the stenopid cleaner shrimp *S. hispidus* is reflected in both the anatomy and volume of neuropil that is dedicated to these inputs (Fig. 2.2D). The bilobed structure of the lateral antenna 1 neuropils is even more pronounced than in the stomatopods, the antenna 2 neuropil is elongated as in the penaeids, and both the lateral antenna 1 neuropil and antenna 2 neuropil have the pattern of fiber bundles running transversely to the long axes of the neuropils. *S. hispidus* has a well-developed olfactory lobe; however the combined volume of the lateral antenna 1 neuropil and the antenna 2 neuropil, measured from silver impregnated serial sections, is nine times larger than the olfactory lobe (Sandeman, unpublished observation). Given the nature of cleaner shrimp behavior, the large increase in the volume of the lateral antenna 1 neuropil in comparison with the malacostracan ground pattern (Fig. 2.2, compare A with F) may reflect an increase in the number and importance of mechanoreceptive and bimodal mechano-chemoreceptors.

The adaptive tendencies in the brains of the species we have examined that are related to exploratory mechanosensory behavior are, like those related to vision and olfaction, largely reflected in the volume, and perhaps complexity, of the neuropils that are associated with modifications to the sensory appendages, be these eyes, olfactory systems or mechano-chemosensory appendages. The malacostracan ground pattern can be recognized in all cases, with the significant exception of the appearance of the accessory lobes in the brains of the eureptantians (Fig. 2.2, compare A with G).

MULTIMODAL INTEGRATIVE BEHAVIOR

The cognitive ability of mammals involves the cortex, an area of the brain with very large numbers of neurons, which receives no direct connections from primary sensory neurons, nor any direct outputs to muscles. It is a "higher" center by virtue of its separation from the "basic" framework that we describe in the Introduction, by relays of interneurons that convey partially processed and coded information that is "multimodal" in that it comes from many sensory systems. Are such higher centers represented in the brains of invertebrates, and if so, do these exhibit adaptations that are related to their behavior?

Long-term and complex behavior patterns appear to be a characteristic of the larger reptantian crustaceans. Among their ranks, we find species that are both the largest and longest living of all arthropods. Although size in itself is not a guarantee for a large brain containing many neurons (e.g., the dinosaurs), at least there is space for a large brain should it be developed. While some arthropods with small brains (honeybees, ants, fruit flies) do indeed carry out complex behavior patterns, large brain size in terms of neuron numbers appears to be associated with a wider variety and flexibility of complex behavior patterns, cephalopod molluscs providing a good example.

Longevity is not necessarily a feature that requires cognitive abilities, an example being the cephalopod mollusk *Octopus vulgaris*, which has a life span of only 12–15 months (Katsanevakis

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and Verriopoulos 2006). Nevertheless an extended life span could well be an important adaptive feature for lobsters that live much longer, providing them with the potential to reproduce over many years and to learn from experience. Their situation is very different from that facing ephemeral mayflies with an adult life span between a few minutes to several days, depending on the species. The adults of these insects have no functional mouthparts and once eclosed, their role is solely to mate, and then they die (Peters and Campbell 1991).

Complex Behavior Patterns

There is no shortage of examples among the malacostracans of complex behavior patterns related to finding nourishment, shelter, territory defense, mating, kin recognition, and brood care. Nevertheless, an area that seems to require a little more than these basic life requirements is that of social interactions among gregarious species during homing, the occupation of common shelters, cooperative behavior during seasonal migration, and the establishment of dominance hierarchies (see also chapter 17 in this volume). It is difficult to conceive such behavior patterns being performed without elements of learning and memory. The spiny lobsters are perhaps the best studied members of the malacostracans in this regard and provide us with some good examples of long-term, complex behavior patterns. Two examples from the spiny lobster *Panulirus argus* are reviewed here; these are the orientation and homing behavior and the complex social behavior involving communal defensive tactics and offshore seasonal migrations.

Orientation and Homing

The spiny lobsters *P. argus* spend the day in shelters, or dens, in reefs. At night, they leave their dens to forage in open areas hundreds of meters away from the reefs, returning before daybreak to their reef and often to the same dens (Herrnkind and McLean 1971). Early tagging studies showed that two lobsters released at distances of 457 m and 3218 m away from their capture site were recaptured at the original site after 4 and 6 days, respectively (Creaser and Travis 1950). Displacement and sonic tracking studies of lobsters carried to about 200 m from their capture site on the reef confirmed the ability of even blinded lobsters to orient accurately toward their home sites (Herrnkind and McLean 1971).

Reef-dwelling spiny lobsters forage widely over the areas surrounding their reef dens and will choose those areas containing the richest source of nourishment, which includes among others, molluscs, arthropods, echinoderms, sponges, and polychaetes (Cox et al. 1997). Reef dens during nonmigratory periods can contain one to several lobsters and are seldom full. Animals within the dens will direct their spiny second antennae toward the entrance. Spiny lobsters do not have offensive appendages such as the large chelipeds of the clawed lobsters and crabs, or the raptorial maxillipeds of the stomatopods. Instead, when approached by a predatory trigger fish or a diver's hand, they will antennate (whip) the intruder with the flagella of the second antennae and then lunge forward to drive off the threat with the strong spines around its base (Atema and Cobb 1980). When a number of lobsters reside together in the same den, they will all direct their antennae toward an intruder and thus present a formidable and thorny barrier (Herrnkind et al. 2001, Briones-Fourzán et al. 2006). Spiny lobsters also stridulate when threatened or attacked by predators (Atema and Cobb 1980).

The path taken by spiny lobsters to their foraging areas and back to their reef dens is not random (Herrnkind et al. 1975, Jernakoff 1987). Spiny lobsters can detect and will orient in relation to the direction of water movement (surge) caused by wave action (Herrnkind and McLean 1971). They are also sensitive to geomagnetic information and walking animals will deviate

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from their chosen course when submitted to artificially generated fields that reverse the natural horizontal, but not vertical, geomagnetic field. Spiny lobsters therefore possess a magnetic field detection system sensitive to field polarity (Lohmann et al. 1995, Boles and Lohmann 2003, chapter 12 in this volume). Nevertheless, in addition to these broad orientational strategies, spiny lobsters returning from foraging areas may also use learned local structural features such as the edges of erosional undercuts along sea grass beds (Cox et al. 1997).

Complex Social Behavior

Adult spiny lobsters are gregarious and tend to aggregate. They are attracted over some distance to conspecifics by chemical cues (Zimmer-Faust et al. 1985) and to dens from which such cues emanate (Zimmer-Faust and Spanier 1987, Childress and Herrnkind 2001). Hence the final stages of homing, at least for those denning with conspecifics, is aided by chemical cues provided by others already in residence (Childress and Herrnkind 2001, Horner et al. 2008). Alternatively, lobsters that are physically damaged through attacks from predators exude chemicals that appear to act as warning cues since approaching lobsters will avoid dens containing such conspecifics (Shabani et al. 2008, Briones-Fourzán et al. 2008).

Panulirus argus is also renowned for the extensive offshore migrations that it undertakes in autumn and that are characterized by a unique "queuing" behavior that has been likened to the flocks that birds form during their long migratory flights. Even in captivity, spiny lobsters have a propensity to line up behind other moving individuals, and the behavior can be seen in animals that return from open nocturnal feeding grounds to their diurnal shelters in the reefs. At the end of the summer, animals in captivity are seen to increase their activity and in circular tanks will form queues and walk along one behind the other for hours. Again this increase in activity has been likened to the "Zugunruhe" that is exhibited by migratory birds shortly before they initiate their migrations.

The behavior of spiny lobsters during their migratory period differs markedly from that seen in nonmigratory periods. Codenning becomes much more common, with some dens completely filled to overflowing. Migratory movements off the Bahamas and the Florida east coast occur during the day (Herrnkind and McLean 1971). Unlike the generally solitary foraging sorties made by nonmigratory spiny lobsters, migrating animals very seldom leave their dens alone. Instead they form "queues" in which the animals line up one behind the other in single file with the second antennae directed at an angle of about 45 degrees lateral to the midline. Constant contact with the individual ahead is maintained with either the inner flagella of the first antennae, anterior walking legs, or second antennae (Herrnkind and McLean 1971).

Migratory queues form in front of the dens when three to five individuals line up and set off together along the migratory pathway. These are followed by other queues that become longer as more individuals join until the den empties, resulting in a number of separate queues all heading in the same direction. Migrating lobsters move away from their protective reefs and over exposed areas of sand and are subject to predation from diurnally active triggerfish. To counter this threat, they exhibit some remarkable defensive behaviors that rely on cooperation between the individuals in the queue. Attacking trigger fish are first confronted with whipping and directed second antennae, followed by the lobsters in the queue forming a "rosette," the entire queue adopting a circular formation from which the second antennae all point outward. Shorter queues that are attacked break from the single file and form a phalanx in which the lobsters form up side by side, facing the threat (Herrnkind et al. 2001).

Migrating lobsters will rest both in areas of reef that lie in their path and in open areas. In reefs, they will aggregate in available crevices and if these fill up, cluster around the entrance.

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In open areas, rosette formation appears to be the usual defensive strategy that is adopted (see Herrnkind et al. 2001 for review).

The social, homing, and migratory behaviors of *P. argus* and other species of spiny lobsters are some of the most complex found in the malacostracans. Depending on visual, olfactory, tactile, and geomagnetic senses, these animals provide a good example of multimodal integrative behavior, and the expectation that their central nervous systems may exhibit some areas beyond the optic ganglia, olfactory lobes, and antenna 2 neuropils to which the primary sensory neurons project.

Higher Integrative Centers

Three neuropil areas in the crustacean brains that are candidates for higher integrative centers can be recognized across species and contain interneurons responding to the selective stimulation of several different sensory systems. These three neuropil areas are the *terminal medullae*, the *hemiellipsoid bodies*, and the *accessory lobes*. To avoid confusion here, the hemiellipsoid body is treated as a separate neuropil and not included as a subsection of the terminal medulla as in some earlier studies (Blaustein et al. 1988). These areas qualify as "higher order" in that they receive information exclusively from second or higher order neurons, contain a large number of small fibered neurons, and have no primary sensory or motor neuron arborizations within them.

The hemiellipsoid bodies and terminal medullae are part of the lateral protocerebrum and often, but not always, located in the eyestalks of those species that have them. There is a strong connection between the olfactory and accessory lobes and the lateral protocerebrum via the large olfactory globular tract. These connections are summarized in Figure 2.5.

The terminal medullae, hemiellipsoid bodies, and accessory lobes are notable for their complexity and the amount of brain space that has been devoted to them, suggesting roles that go beyond simple reflexive behavior and that may involve more sophisticated processing related to orientation within the environment during homing or migration, recognition of suitable mating partners, and social interactions. All of these require aspects of learning and memory (see chapter 19 in this volume).

Terminal Medulla

The terminal medulla is a collection of smaller neuropil areas that are partly confluent with one another but that can be anatomically distinguished along their peripheral borders into a series of lobes. The neuropil structure within these areas is tangled, has a heterogeneous appearance, and contains both coarse and fine fibers. As such it resembles many other areas in the brain. There are eleven neuropil areas in the terminal medulla in both *Procambarus clarkii* and *Panulirus argus*, excluding the two that make up the hemiellipsoid body (Blaustein et al. 1988). Tracts between some terminal medulla neuropils and the optic ganglia, and from others to the olfactory and accessory lobes, suggest that the terminal medulla is a center in which a considerable amount of interaction between the olfactory and visual input occurs. This is supported by physiological studies in which electrical recording from, and labeling of, individual neurons that project to neuropils within the terminal medulla. Those with somata in the trito-, deuto-, or median protocerebrum and projections to the terminal medulla exhibit sensitivities to stimuli that range from unimodal chemical, through bimodal chemical and mechanical, to multimodal including chemical, mechanical, and photic. Neurons with their somata in the terminal medulla and that had morphologically complex branching were excited by chemicals applied to the first antennae but inhibited by tactile or visual stimuli (Derby and Blaustein 1988).

The terminal medulla of *B. latro* is, like in all the other species so far examined, not geometrically arranged into columns or layers and is connected to the optic neuropils through the axons

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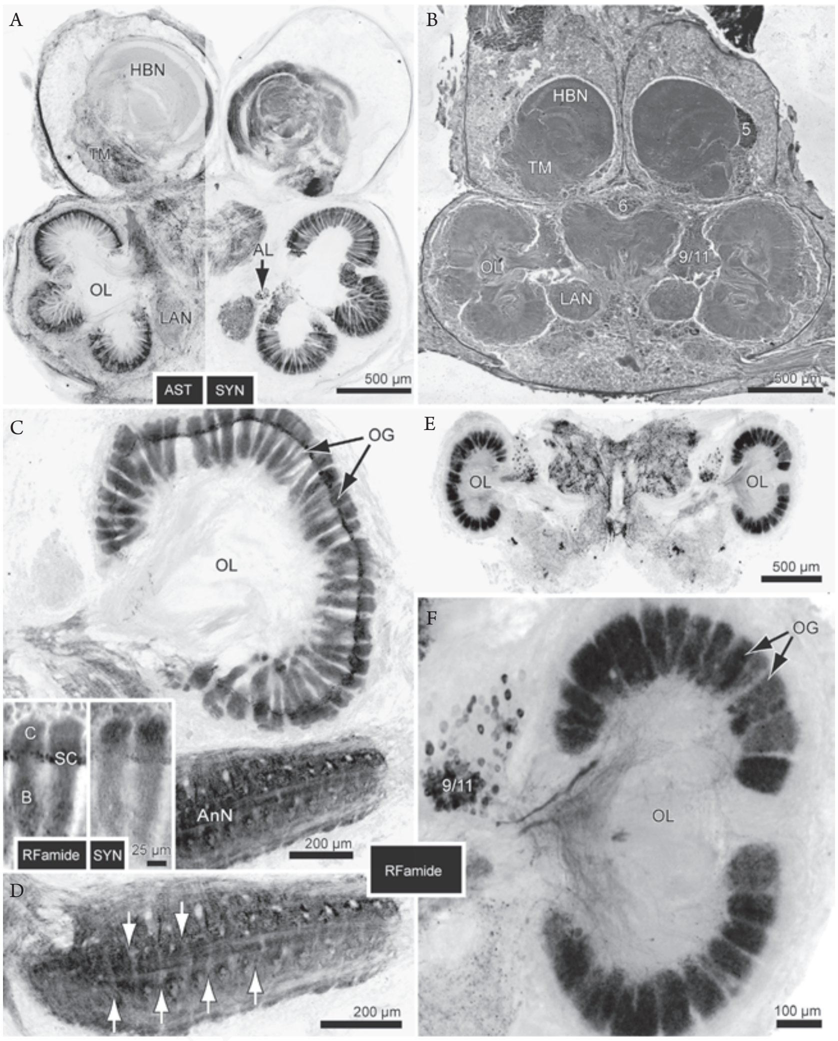

Fig. 2.5.
(A and B) Brain of the giant robber crab *Birgus latro* (Anomura; from Krieger et al. 2010, with permission from BioMed Central). (A) A single vibratome section (100 µm) double labeled against allatostatin-like immunoreactivity (AST, left) and synapsin immunoreactivity (SYN, right) to highlight the hemiellipsoid body (HBN), terminal medulla (TM), olfactory lobe (OL), accessory lobe (AL), and lateral antenna 1 neuropil (LAN) (B) A silver-impregnated microtome section (10 µm) showing the same neuropil areas as in A and the location of somata clusters 5, 6, 9, and 11. (C and D) Marine hermit crab *Pagurus bernhardus* (Anomura). (E and F). Shore crab *Carcinus maenas* (Brachyura). Immunolocalization of RF-amide-like neuropeptides on vibratome sections of the brains (from Harzsch et al. 2011, with permission from Elsevier). (C) *P. bernhardus*, right olfactory lobe (OL; anterior is toward the top) with olfactory glomeruli (OG) and antenna 2 neuropil (AnN). Insets: higher magnification of olfactory glomeruli showing RF-amide-like (left) and synapsin-like immunoreactivity (right) to demonstrate their subdivision into cap (C), subcap (SC) and base (B). (D) *P. bernhardus*, transverse segmentation (arrows) of the antenna 2 neuropil. (E) *C. maenas*, horizontal section showing an overview over the brain. (F). *C. maenas*, higher magnification of the right olfactory lobe (OL) to show the radial arrangement of the cone-shaped glomeruli (OG). C–F from Harzsch et al. 2011, with permission from Elsevier. Scale bars: A, 500 µm; B, 100 µm; C, 200 µm; insets, 25 µm; D, 200 µm; E, 500 µm, F, 100 µm.

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in the optic tract. The medial area of the terminal medulla receives projections from the lobula and the lobula plate which are ontogenetically derived from the terminal medulla and so may be considered to be part of the lateral protocerebrum rather than being optic neuropils. The terminal medulla of *B. latro* also receives a significant deutocerebral input from the olfactory lobe via the axons in the olfactory globular tract (Krieger et al. 2010).

Hemiellipsoid Bodies

The hemiellipsoid bodies are separated into two areas in the spiny lobsters (I and II, Blaustein et al. 1988), the crayfish *P. clarkii* and *O. rusticus* (neuropil I and II, Sullivan and Beltz 2001), the crayfish *C. destructor* (HBI and HBII, Sullivan and Beltz 2005), and the clawed lobster *Homarus americanus* (core and cap neuropil, Sullivan and Beltz 2001). While anatomically probably homologous, there are some differences in structure between the crayfish and the lobsters and also differences in the areas that are targeted by the axons of the projection neurons that ascend from the deutocerebrum. In crayfish, the hemiellipsoid bodies are targeted by projection neurons from the accessory lobes and not from the olfactory lobes (Mellon et al. 1992a, 1992b, Sullivan and Beltz 2001, 2005), whereas those from the olfactory lobes target primarily the terminal medullae. There is therefore very little overlap of the input from these two areas at the level of the lateral protocerebrum. Furthermore, in the crayfish *C. destructor*, the projection neurons from the cortex and medulla of the accessory lobes project separately to the inner (HBII) or outer (HBI) neuropils of the hemiellipsoid bodies. In the clawed lobster *H. americanus*, however, while there is still a pronounced separation of the projections from the accessory lobes and the olfactory lobes to the cap neuropil (I) and the core neuropil (II), the cap neuropil also receives branches from the olfactory lobes that sweep over the outer surface of the cap and form a layer within the cap (Sullivan and Beltz 2001). This is interesting in terms of the situation that is found in the hemiellipsoid bodies of the coconut crab *B. latro*, as described later in this section.

Electrophysiological and anatomical investigation of the hemiellipsoid bodies in the crayfish *C. destructor* and *P. clarkii* brought a class of interneurons to light that are local to the hemiellipsoid bodies and that branch extensively in the two neuropil areas of the hemiellipsoid bodies (Mellon et al. 1992a, 1992b, Mellon and Alones 1997, Mellon 2000, McKinzie et al. 2003). There are about 200 of these so-called parasol cells that exhibit various patterns of continuous activity that are modified by olfactory, tactile, and visual stimuli (Mellon and Alones 1997, Mellon and Wheeler 1999, Mellon 2000, 2003) (Fig. 2.6). The somata of these cells are located ventral to the terminal medulla, and their primary neurites project to and arborize within either the HBI or HBII neuropils (McKinzie et al. 2003). The parasol cells, despite the common location of their somata, can therefore be considered to represent two classes of neurons depending on the nature of their inputs. In addition to the dendritic projection of the parasol cells in either of the HB neuropils, an axon extends to arborize in the terminal medulla (McKinzie et al. 2003).

The hemiellipsoid bodies in *B. latro* are large, located medially in the brain and not in the eyestalks, and separated into three distinct areas, reflecting to some extent the situation in *H. americanus* (Sullivan and Beltz 2001). The structure of the hemiellipsoid body in *B. latro* resembles the layers of an onion (Krieger et al. 2010), with a peripheral and hemispherical cap neuropil enclosing two neuropil areas CO1 and CO2, which may be homologous with the neuropil areas I and II of the crayfish, or, depending on the status of the cap neuropil in the lobster may represent a subdivision in *B. latro* or the *H. americanus* core neuropil. At present, details of the pathways between these areas in *B. latro* and the deutocerebral neuropils that could indicate homologies are not yet available. Such information would indeed be of interest in relation to the changing functions of the olfactory lobe and hemiellipsoid body that may have compensated for the absence of large accessory lobes in *B. latro*, or represent a shift in emphasis of higher order neuropils and processing capability toward olfactory instead of tactile inputs.

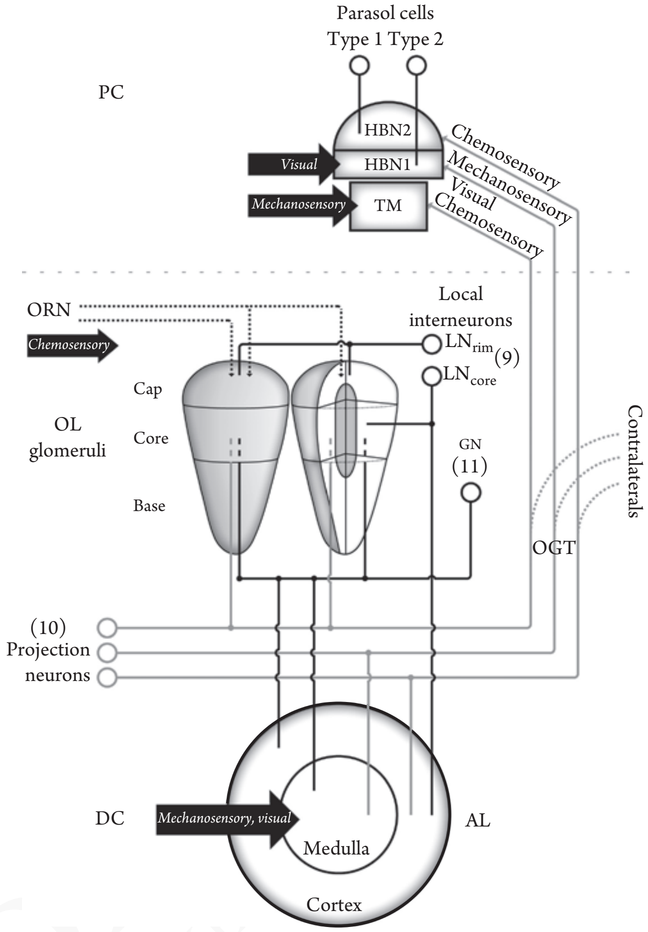

Fig. 2.6.
Summary of the known connections between the lateral deutocerebral and lateral protocerebral neuropils of crayfish and clawed lobsters. The olfactory receptor neurons are the only primary sensory afferent input, and these end in the glomeruli of the olfactory lobe. The accessory lobe, terminal medulla, and hemiellipsoid body receive chemical, mechanosensory, and visual input via interneurons. Olfactory information is relayed from the olfactory lobe to the cortex of the accessory lobe, to the terminal medulla, and to the outer neuropil of the hemiellipsoid body. Visual information reaches the medulla of the accessory lobe and the inner neuropil of the hemiellipsoid body. Mechanosensory information is directed to the medulla of the accessory lobe, to the terminal medulla, and to the inner neuropil of the hemiellipsoid body. Identified local interneurons are associated with the olfactory and accessory lobes (local olfactory interneurons, dorsal giant neuron, and midbrain olfactory interneurons) and with the hemiellipsoid bodies (parasol cells). (Based on the results of Derby and Blaustein 1988, Blaustein et al. 1988, Sandeman and Sandeman 1994, Mellon and Alones 1995, 1997, Sandeman D. et al. 1995, Sullivan and Beltz 2001, 2005, McKinzie et al. 2003, Mellon 2007). Abbreviations: AL, accessory lobe; DC, deutocerebrum; GN, dorsal giant neuron; HBN1-2, subdivision of the hemiellipsoid body neuropil; LN, local interneuron; OGT, olfactory globular tract; ORN, olfactory receptor neuron; PC, protocerebrum; TM, terminal medulla.

Accessory Lobes

The spiny lobsters have large and complex accessory lobes. These lie medial to the olfactory lobes and have three layers of glomerular neuropil (Blaustein et al. 1988) in which those in the outer layer are columnar, while those in the inner two layers are spherical (Sandeman et al. 1992, Wachowiak et al. 1996, Schmidt and Ache 1997). The accessory lobes of the astacids have two concentric layers of glomerular neuropil that are all spherical in crayfish (Sandeman D. et al. 1995). In the clawed lobster *H. americanus*, the glomeruli in the outer layer are columnar like those found in the spiny lobsters, and spherical in the inner layer (Helluy et al. 1993).

The accessory lobes receive no primary afferent inputs, and the input from the olfactory lobes, via local interneurons, terminates predominantly in the cortical areas (Sullivan and Beltz 2005). The accessory lobes in *C. destructor* also receive inputs from a large number of interneurons in the deutocerebral commissure. Intracellular electrical recordings and subsequent labeling of these interneurons with neurobiotin revealed five anatomical classes of interneurons. These have unilateral inputs located in different areas in the proto- or deutocerebrum or bilateral inputs from the tritocerebrum, and all have bilateral outputs ending in the glomeruli of the left and right accessory lobes. Information from olfactory, visual, and tactile inputs are represented in the axons of the interneurons of the deutocerebral commissure (Sandeman D. et al. 1995), and ultrastructural studies of labeled deutocerebral commissure interneurons confirmed them to be presynaptic to elements within the accessory lobe (Sandeman R. et al. 1995).

The accessory lobes in *B. latro* on the other hand, although containing small spherical glomeruli like those in the crayfish, are very small and do not appear to be connected to each other or to other areas of the brain via a deutocerebral commissure. Given their very small volume, it is difficult to see how they could play a dominant role in the olfactory processing in these animals such as that proposed for the crayfish or lobsters. The emphasis on the olfactory lobes and the hemiellipsoid bodies in *B. latro*, and the extreme reduction of the accessory lobes, suggest a shift in the balance of processing power. Spiny lobsters, and some crayfish with equally long second antennae, have significantly larger accessory lobes compared with those species relying on olfactory and visual senses. Hence the accessory lobes could be integrative areas also serving exploratory mechano-chemosensory behavior (see Conclusions).

Evolution of the Malacostracan Higher Integrative Centers

Early carcinologists identified the olfactory lobes, accessory lobes, and lateral protocerebrum as areas where considerable evolutionary transformation has taken place during phylogeny of Malacostraca. Olfactory lobes were most likely present in the ground pattern of Malacostraca and linked to the lateral protocerebrum by the protocerebral tract (Fig. 2.2A). In the Decapoda, the projection neurons probably equally innervated both the terminal medullae and the hemiellipsoid bodies, and contralateral projections of the olfactory globular tract were present as in the original malacostracan ground pattern (Fig. 2.7).

This configuration could be homologous to that of basal insects, which lack mushroom bodies (Farris 2005, Galizia and Rössler 2010). Their antennal lobes (homologs of the malacostracan olfactory lobes) are linked to the lateral horn in the protocerebrum (a homolog of the malacostracan lateral protocerebrum), but the antennocerebral tract is uncrossed (Galizia and Rössler 2010). During evolution of the Pterygota, the functional need for more sophisticated olfactory processing arose to cope with airborne odors, and insects developed mushroom bodies (only the peduncles in the beginning) to provide an adequate neuronal substrate. They also evolved additional projection neuron tracts that link the antennal lobes to the secondary olfactory areas in the protocerebrum (Fig. 2.7; Galizia and Rössler 2010, Strausfeld 2012).

The Malacostraca appear to have followed a different pathway to increase their capacity for analyzing olfactory stimuli. In the Reptantia, the accessory lobe, an evolutionary novelty, arose. Most of the projection neurons associated with the accessory lobes in Reptantia target the hemiellipsoid bodies, whereas those from the olfactory lobe are connected predominantly to the terminal medullae (Sullivan and Beltz 2001, 2005).

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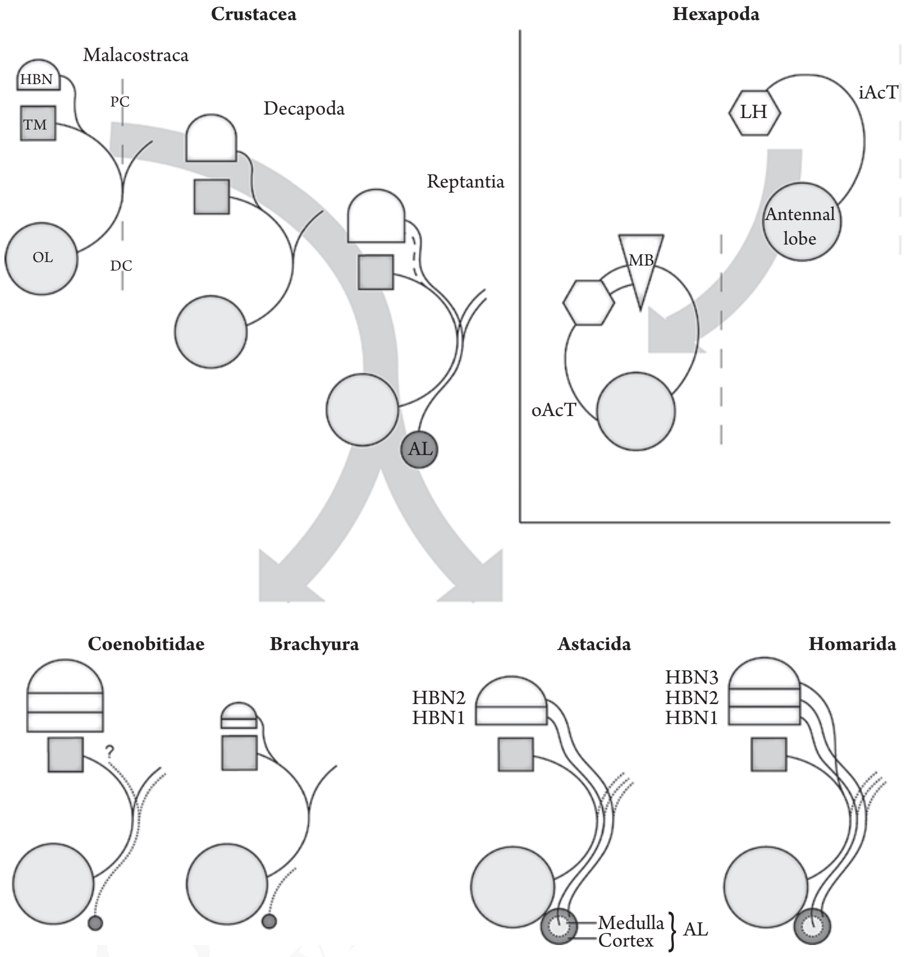

Fig. 2.7.
Hypothesis on the evolution of the malacostracan and insect higher integrative centers (for further details see text). Abbreviations: AL, accessory lobe; DC, deutocerebrum; HBN, hemiellipsoid body; HBN1-3, subdivision of the hemiellipsoid body neuropil; iAcT, inner antennocerebral tract; LH, lateral horn; MB, mushroom body; oAcT, outer antennocerebral tract; OL, olfactory lobe; PC, protocerebrum; TM, terminal medulla. Question mark denotes uncertainty about the connection.

The accessory lobes first appeared in early Reptantia, then lost their presumed role as secondary olfactory centers in Brachyura and Anomura. The development and then loss of the accessory lobes is difficult to resolve in terms of lifestyle and habitat although there are differences between the groups that may provide some clues. Many of the Achelata, Homarida, and Astacida are either nocturnal or crepuscular and have well developed second antennae and good mechanosensory abilities. Many of the Brachyura on the other hand have well-developed visual systems but the second antennae are often relatively short. Many are active both at night and during the day. In terms of the proportional sensory modalities, the animals would appear to rely on a combination of olfactory and visual inputs. The Thalassinida represents an intermediate stage: *Callianassa australiensis* has enlarged hemiellipsoid bodies that are located medially in the brain and accessory lobes that, while smaller than those of the Astacida, are linked to one another across the brain by a well-developed deutocerebral commissure (Sandeman and Scholtz 1995).

The Coenobitidae in this context is an interesting case because this group of terrestrial hermit crabs, which includes the genus *Coenobita* and the monospecific genus *Birgus*, has secondarily emphasized the olfactory pathway (Harzsch and Hansson 2008, Krieger et al. 2010). There is good behavioral and physiological evidence for a sense of aerial olfaction in members of the Coenobitidae (review Hansson et al. 2011), and their olfactory lobes are large. The hemiellipsoid bodies are also characteristically layered and large in comparison with those found in most other decapods (Fig. 2.2).

In summary, it appears that in Insecta and Malacostraca, we find three different strategies to increase the analytical and integrative need to match the shifting proportion of sensory inputs (Fig. 2.7). This could be shared between visual, olfactory, and mechanosensory or dominated by any one, or a pair of inputs. In terms of the chemosensory input: (1) introduce a new neuropil in the protocerebrum and hook it up to the central olfactory pathway (Pterygota), (2) introduce a new neuropil in the deutocerebrum (Reptantia) and hook it up to the central olfactory pathway, and (3) inflate the existing neuronal substrate of the central olfactory pathway and elaborate its architecture (Coenobitidae).

FUTURE DIRECTIONS

Classical methods have provided us with a basic morphological and physiological framework in which to understand the brains and behavior of some of the larger decapod crustaceans. Advances beyond this depend on the development and application of new methods and techniques of which there are already an exciting range from which to choose. For example, miniaturization of video and radio tracking devices as well as GPS-based telemetry systems now allow ethologists to move into the field and beyond the observation of animals confined in enclosures or aquaria. Developments in immunocytochemistry provide chemical snapshots of the ebb and flow of a host of neurochemical agents that can be related to behavioral activities. The introduction of laser scanning microscopes and fluorescent dyes has considerably extended the resolution of conventional microscopes. Three-dimensional reconstructions of image stacks either obtained from confocal microscopy or serial sections add significant new possibilities to neuroanatomical analysis, enabling comparative and evolutionary neuroanatomists to rapidly screen and explore morphological variations in many different species. Recent advances in computer tomography permit the relatively noninvasive observation of brain chemistry in real time, thus freeing physiologists from the need to impale living tissues and interpret the neuronal chemistry from associated electrical signals. Such innovative approaches may be expected to advance our understanding in terms of the phylogenetic relationships within the Crustacea as a whole and also of the roles played by the “difficult” areas of the crustacean brains, such as the hemiellipsoid bodies and the accessory and olfactory lobes. These, by virtue of their sheer complexity and large numbers of small neurons contained within them, have resisted the attempts of physiologists to unravel their connectivity or provide a coherent explanation of their functional roles in crustacean behavior. It is here that the application of the new wave of technologies could have the greatest impact.

CONCLUSIONS

Brain and Behavior in the Malacostracans

In comparing the neuropils within the Malacostraca, one feature seems to be clear: the high level of conservation of the characteristic anatomical features that we use to recognize the

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neuropils. This may be explained to some extent by a consideration of the roles of these neuropils and the type of modality that processing within them extracts from the receptor cells that project to them. In visual systems, the information from an array of photoreceptors that can merely respond to light intensity is translated into the important properties that constitute vision, namely extraction from the photoreceptor array of the contrasting boundaries that go to make up objects and the interpretation of the direction and velocity of the movement of this object. To achieve this, the information from the photoreceptors passes through layers of elements where specific lateral interactions occur and where the nature of the information that is extracted gradually becomes more sophisticated, changing from mere light intensity to the detection of differences between areas, the sensitivity only to edges or lines in particular directions, and the direction and velocity of motion. This is the case in all visual systems, both vertebrate and nonvertebrate. Hence, it is perhaps no surprise to find that despite the vast difference in the visually guided behavior patterns of stomatopods and swimming crabs on the one hand and that of the isopods on the other, the actual anatomical characteristics of the layered optic neuropil appear very similar. The essential differences in terms of the abilities of the two groups presumably lie in the numbers and in the tuned responses of the individual neurons that are contained in the various layers. The differences between the animals' visual systems are therefore not qualitative (they are all capable of detecting the direction and velocity of a moving edge) but quantitative in terms of their precision.

This argument also applies to the olfactory systems. Olfactory receptor cells in many animals are supplied with unique molecular receptors that constitute the first filter in the system in selecting specific odors, much like photoreceptors are able to selectively respond to different wavelengths of light. There is a precise projection of these cells, as in the visual system, to discrete areas of the olfactory lobe neuropil where specific processing will extract the chemical information that is meaningful for the animal. As in the case of the visual system, the structure of malacostracan olfactory systems is highly conserved, consisting of tight areas of neuropil or glomeruli. From these areas that receive the primary afferent inputs, there are trunks of large numbers of fine axons, the projection neurons, that extend to a second area of neuropil in the protocerebrum, the hemiellipsoid bodies, which in some species (*B. latro*, for example) have layers of laterally projecting neurons.

The extraction of the relevant information in exploratory mechano-chemosensory systems may be equally complex in comparison with vision and olfaction. The antenna 2 neuropil in animals with long flexible second antennae is geometrically structured and may provide the substrate for the spatiotopic location of antennal stimulation.

If animals are employing their second antennae, and other appendages (legs) for the active mechano-chemosensory exploration of their surroundings, this would require a high degree of central integration incorporating not only the comparison of motor commands with the ensuing sensory responses, but also including visual and olfactory inputs. Where is the relevant higher order neuropil for this system? Perhaps the assignment of the accessory lobes purely to the olfactory pathway is an oversimplification and its real function extends to the reception of the wide range of information gathered during the active examination of objects and the surroundings, the evaluation of this in relation to previous experience and the generation of appropriate action. Functional connections between the antenna 2 neuropils, the visual system, the olfactory lobes, and the accessory lobes have been described in *C. destructor* (Sandeman D. et al. 1995) and are highly likely to be also present in other species with accessory lobes.

Support for a highly integrative and behaviorally relevant role for the accessory lobes has come from a recent exploration of drug dependency which used crayfish as a model system: an

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alteration of the c-Fos mRNA expression in the accessory lobe of crayfish has been associated with a conditioned cocaine-induced reward. The remarkable aspect of this study in the context of the function of the accessory lobes is that textural information from the substratum on which the animals were placed was coupled with the cocaine conditioning. This suggests that the mechanoreceptive differences in the substratum (pebbled or smooth) detected by the walking legs are relayed to the accessory lobes, which in turn are implicated in the behavioral changes associated with the drug administration (Nathaniel et al. 2012).

Further evidence for the multifunctionality of the accessory lobes comes from the discovery of a neurogenic niche associated with the accessory lobes of many of the reptantians (Bazin 1970, Sandeman et al. 2011, chapter 7 in this volume). Best described in the crayfish *P. clarkii* (Benton et al. 2011, Beltz et al. 2011) and spiny lobster *P. argus* (Schmidt 2007, Schmidt and Derby 2011), this system generates new local and projection neurons throughout the life of the adult animals, and these new neurons are incorporated into the olfactory pathway (see chapter 7 in this volume).

Multimodality, Convergence, and Cognition

Spiny lobsters and coconut crabs are both large and long-lived species of crustaceans, and both exhibit behaviors that are complex and of long duration and in which a particular sequence is essential in order for the ultimate behavioral aim to be achieved. Migrations must be initiated and directed, intermediate localities and appropriate releasers recognized and acted on, and finally home journeys initiated, undertaken, and home localities found.

Behaviors of this kind, with the unpredictable intermediate events that could occur during the completion of the task, cannot be undertaken without some level of learning and recognition of a particular constellation of olfactory, visual, tactile, and geomagnetic stimuli. The actual task of combining these features into a unique set that can be matched with some preexisting "image" is no different for a homing spiny lobster or crab than that confronting any "higher" organism equipped with a cortex.

To combine a set of various sensory stimuli into some unique neuronal picture requires the presence of multimodal neurons that will respond to a particular set of inputs that may occur in a particular sequence and at particular levels of intensity. Certainly there are many examples of these kinds of neurons at all levels in the brains of the crustaceans and particularly in the accessory lobe, hemiellipsoid body, and terminal medulla. These may be responsible for a highly selective filtering of sets of environmental inputs and trigger an appropriate behavior. In other words, simple convergence, given adequate and perhaps tuned preset thresholds, could do the job, and the brain areas containing these are represented in the crustacean central nervous system (Fig. 2.6).

There are, however, some difficulties with such a simple model. In a consideration of how multiple sensory inputs are integrated in the mammalian cortex, Tonini et al. (1998) point out that convergence is unlikely to be the predominant mechanism because no "master" brain area has been identified and there are so many possible combinations of stimuli, each of which would need its own master area, that there are not even enough single neurons available to cover the combinatorial explosion that would ensue (and certainly not in the relatively small brains of the arthropods). The same authors point out that convergence will not allow the flexibility needed to respond to novel stimuli, and in the context of a coconut crab finding its way to the coast to mate, or a spiny lobster migrating offshore, it is certain that the detailed features of the landscape through which they make their way are not going to be precisely the same in subsequent migrations.

Instead of simple convergence of multimodal inputs, it seems that a more likely solution would include interactions between functionally segregated brain areas that act to synchronize

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the ongoing activity of groups of neurons (Tonini et al. 1998). A feature of such models is that target cells are not driven by such connections but that the timing of their firing is modulated. In this regard, the parasol cells in the hemiellipsoid body of the crayfish provide an interesting parallel (Mellon and Wheeler 1999, Mellon 2000, McKinzie et al. 2003). These neurons fire spontaneously, exhibit particular and individual patterns, and, most important for the comparison with the cortex model, these patterns of activity are *modulated* rather than directly driven by their multimodal receptor inputs. They could therefore represent the morphological substrate for the "reentrant" requirement of the cortex model, in which parallel and collateral interactions between separate brain areas occur. Furthermore, the cortex model, which satisfies many experimental findings, contains elements such as "functional clusters" that are defined as a "set of brain regions that interact much more strongly with each other than with the rest of the brain" (Tonini et al. 1998). The accessory lobe, the hemiellipsoid body, and terminal medulla in the malacostracans are candidates for such functions and worth exploring in this context.

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7. Ausweis der Eigenanteile bei einer kumulativen Dissertation

I Kenning M, Harzsch S (2013) - Brain anatomy of the marine isopod *Saduria entomon* Linnaeus, 1758 (Valvifera, Isopoda) with special emphasis on the olfactory pathway.

Experimente, Recherche, Abbildungen und Bildtafeln, Textverfassung (90 %)

II Kenning M, Lehmann P, Lindström M, Harzsch S (2015) - Heading which way? Y-maze chemical assays: not all crustaceans are alike.

Experimente, Recherche, Abbildungen und Bildtafeln, Textverfassung (80 %)

III Kenning M, Lindström M, Harzsch S (eingereicht) - Randomness on the trial – Food search patterns in a marine Isopod.

Experimente, Recherche, Abbildungen und Bildtafeln, Textverfassung (80 %)

IV Sandeman D, Kenning M, Harzsch S (2014) - Adaptive trends in malacostracan brain form and function related to behaviour.

Recherche, Abbildungen und Bildtafeln, Textverfassung (30 %)

Unterschrift Betreuer Unterschrift Promovend

8. Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

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