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BDS

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The Journal of the British Dragonfly Society, published twice a year, contains articles on Odonata that have been recorded from the United Kingdom and articles on European Odonata written by members of the Society.

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Editor:
Peter Mill
8 Cookridge Grove
Leeds, LS16 7LH
email: gpmill@supanet.com

Secretary:
Henry Curry
23 Bowker Way
Whittlesey
Peterborough, PE7 1PY
email: secretary@british-dragonflies.org.uk

Librarian / Archivist:
David Goddard
30 Cliffe Hill Avenue
Stapleford
Nottingham, NG9 7HD
email: david.goddard8@ntlworld.com

Membership Secretary
Lynn Curry
23 Bowker Way
Whittlesey, Peterborough, PE7 1PY
email: membership@british-dragonflies.org.uk

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Cover illustration: Second instar larva of Sympetrum striolatum. Photograph by Steve Cham.

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Authors are asked to study these instructions with care and to prepare their manuscripts accordingly, in order to avoid unnecessary delay in the editing of their manuscripts.

- Word processed manuscripts may be submitted in electronic form either on disk or by e-mail.
- Manuscripts should be one and a half spaced, on one side of the page only and with margins at least 25mm on both sides and top and bottom. Footnotes should be avoided.
- Use of these terms is acceptable: ‘exuvia’ for cast skin (plural: ‘exuviae’); ‘larva’ (instead of ‘naiad’ or ‘nymph’); ‘prolarva’ to designate the first larval instar.
- Dates in the text should be expressed in the form: 24 July 2010.
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- The legend for each table and illustration should allow its contents to be understood fully without reference to the text.

Please refer to a recent issue of the journal for further style details.

SCIENTIFIC AND ENGLISH NAMES OF BRITISH ODONATA

ZYGOPTERA	DAMSELFLIES
Calopteryx splendens	Banded Demoiselle
Calopteryx virgo	Beautiful Demoiselle
Ceriagrion tenellum	Small Red Damselfly
Chalcolestes viridis	Willow Emerald Damselfly
Coenagrion armatum	Norfolk Damselfly
Coenagrion hastulatum	Northern Damselfly
Coenagrion lunulatum	Irish Damselfly
Coenagrion mercuriale	Southern Damselfly
Coenagrion puella	Azure Damselfly
Coenagrion pulchellum	Variable Damselfly
Coenagrion scitulum	Dainty Damselfly
Enallagma cyathigerum	Common Blue Damselfly
Erythromma najas	Red-eyed Damselfly
Erythromma viridulum	Small Red-eyed Damselfly
Ischnura elegans	Blue-tailed Damselfly
Ischnura pumilio	Scarce Blue-tailed Damselfly
Lestes barbarus	Southern Emerald Damselfly
Lestes dryas	Scarce Emerald Damselfly
Lestes sponsa	Emerald Damselfly
Platycnemis pennipes	White-legged Damselfly
Pyrrhosoma nymphula	Large Red Damselfly
Sympecma fusca	Winter Damselfly
ANISOPTERA	DRAGONFLIES
Aeshna affinis	Southern Migrant Hawker
Aeshna caerulea	Azure Hawker
Aeshna cyanea	Southern Hawker
Aeshna grandis	Brown Hawker
Aeshna juncea	Common Hawker
Aeshna mixta	Migrant Hawker
Anaciaeshna isoceles	Norfolk Hawker
Anax ephippiger	Vagrant Emperor
Anax imperator	Emperor Dragonfly
Anax junius	Green Darner
Anax parthenope	Lesser Emperor
Brachytron pratense	Hairy Dragonfly
Cordulegaster boltonii	Golden-ringed Dragonfly
Cordulia aenea	Downy Emerald
Crocothemis erythraea	Scarlet Darter
Gomphus flavipes	Yellow-legged Club-tail
Gomphus vulgatissimus	Common Club-tail
Leucorrhinia dubia	White-faced Darter
Leucorrhinia pectoralis	Large White-faced Darter
Libellula depressa	Broad-bodied Chaser
Libellula fulva	Scarce Chaser
Libellula quadrimaculata	Four-spotted Chaser
Orthetrum cancellatum	Black-tailed Skimmer
Orthetrum coerulescens	Keeled Skimmer
Oxygastra curtisii	Orange-spotted Emerald
Pantala flavescens	Wandering Glider
Somatochlora arctica	Northern Emerald
Somatochlora metallica	Brilliant Emerald
Sympetrum danae	Black Darter
Sympetrum flaveolum	Yellow-winged Darter
Sympetrum fonscolombii	Red-veined Darter
Sympetrum pedemontanum	Banded Darter
Sympetrum sanguineum	Ruddy Darter
Sympetrum striolatum *	Common. Darter *
Sympetrum vulgatum	Vagrant Darter

* Includes dark specimens in the north-west formerly treated as a separate species, *Sympetrum nigrescens* Highland Darter

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Observations of egg development, hatching and early instars of *Sympetrum striolatum* (Charpentier) Common Darter

Steve Cham

2 Hillside Road, Lower Stondon, Henlow, Bedfordshire, SG16 6LQ. stevecham1@aol.com

Abstract

Sympetrum striolatum Common Darter is a common and widespread species across the United Kingdom with the adult and larval stages well studied. The egg stage however is less well known. Observations on egg development, hatching and early instars during late summer indicate a high level of fertilisation and survivorship under controlled conditions.

Introduction

After successful copulation, *Sympetrum striolatum* Common Darter typically oviposit in tandem over open water, often in places with submerged plants just below the surface (Brooks *et al*., 2014). After tandem, the pair separate and the female will usually continue ovipositing, sometimes with the male hovering nearby guarding the female. Eggs are deposited in clusters that sink through the water attaching to the first substrate they come into contact with. Egg development is one of the least studied stages and by collecting eggs from a female the development and hatching can be observed under controlled conditions. Egg survivorship and subsequent hatching of the prolarva and stadium 2 larva determines the start of the life cycle. This study documents some observations of egg development, hatching and survivorship of *S. striolatum*.

Material and Methods

Tandem pairs of *Sympetrum striolatum* were visiting a recently constructed garden pond during August 2017. They oviposited in areas in full sun where submerged plants were at, or just below, the water surface (Plate 1A.1B). On 2 September 2017 at 12.45 one tandem pair was ovipositing over water at the pond. It was caught in flight with a kite net and subsequently separated in the confines of the net. The male was released and the female restrained by holding

A

B

Plate 1A, 1B. A tandem pair of *Sympetrum striolatum*, Common Darter, ovipositing in tandem. A) The eggs and a water droplet can be seen at the tip of the female's abdomen, B) The female depositing the eggs into the water.

the wings. Using a series of up and down dipping motions, to mimic the actions of the male during exophytic oviposition, the female was induced to shed eggs into a small shallow petri dish that had been pre-filled with pond water to a depth of approximately 6mm. The eggs settled in clusters, adhering to the base of the dish. The female was then released unharmed.

The eggs were observed under a stereo microscope at magnifications between 60-650X. The illumination during observations was provided by a twin gooseneck fibre optic light source to avoid heating the water in the dish. Altogether 449 eggs were obtained and they were measured with a micrometer accurate to 0.1mm. They were monitored regularly each day under the microscope for signs of development. The temperature of the water was recorded using a digital thermometer probe and then repeated at intervals throughout the study. The dish was kept in a north east facing room inside the house that was not subject to extremes of temperatures. Temperatures were consistently around 19.7°C during the days and evenings throughout.

The eggs were observed to the point of hatching and the prolarvae and stadium 2 larvae were observed for a further seven days. Unhatched eggs were observed for a further 42 days after this for signs of late development or decomposition. Due to the lack of a food source small enough for the stadium 2 larvae they were released back into the garden pond.

Results

The eggs of *Sympetrum striolatum* are chalky white when first deposited (Plate 2) and change to a golden brown over the following 12 hours (Plate 3). They were ovoid in shape, measuring 0.55 x 0.40mm. Of the 449 eggs collected only two did not change colour; they remained white and were considered to be unfertilised.

Within one hour of the eggs being collected it was evident that a gel-like substance was forming around them that subsequently merged overnight to form a continuous amorphous mass around all the eggs. As the amorphous gel mass expanded it became more elastic and difficult to divide and appeared to lose its stickiness when touched with a mounted eyelash probe. The gel and encapsulated eggs were firmly adhered to the glass petri dish and resisted mild attempts to remove it by prodding with a fine mounted needle.

Over the first eight days globules of yolk could be seen inside the eggs (Plate 4). The two white, unfertilised eggs developed a white growth within two days, probably a fungal infection, causing them to split. The white mass expanded

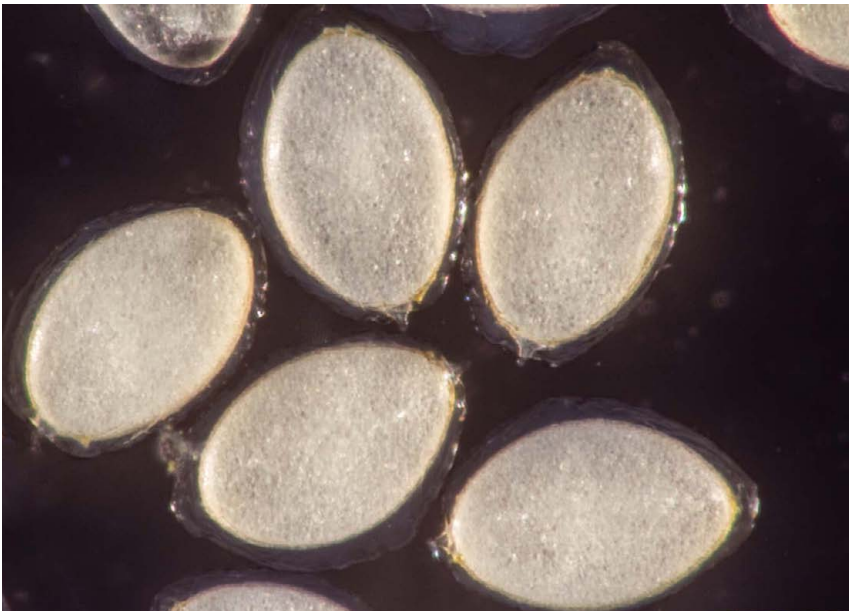


Plate 2. Freshly deposited eggs one hour after oviposition, showing the amorphous yolk before darkening of the eggshell. (Scale bar = 0.5mm).

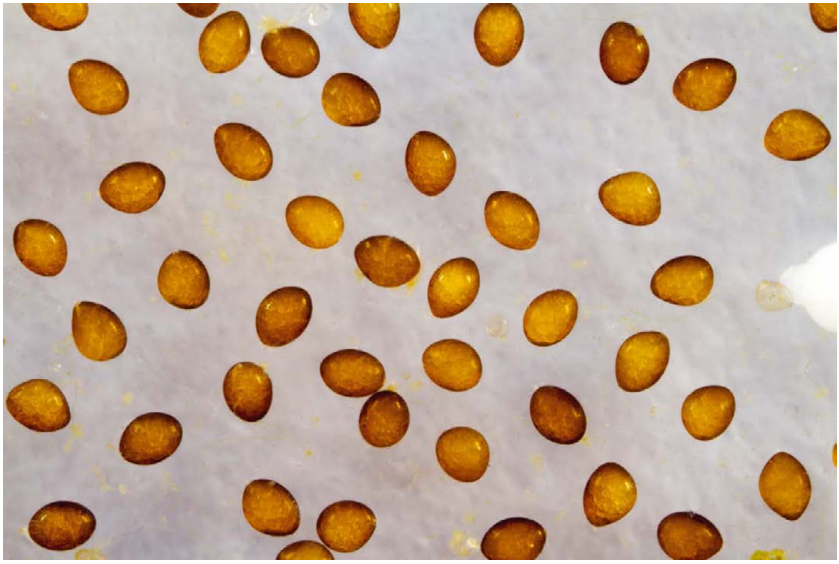


Plate 3. The eggs showing the darkening of the eggshell. They are attached to the bottom of a glass dish. The ovoid eggs are 0.40 x 0.55mm. The white mass at the far right edge is an unfertilised egg that has a fungal infection.

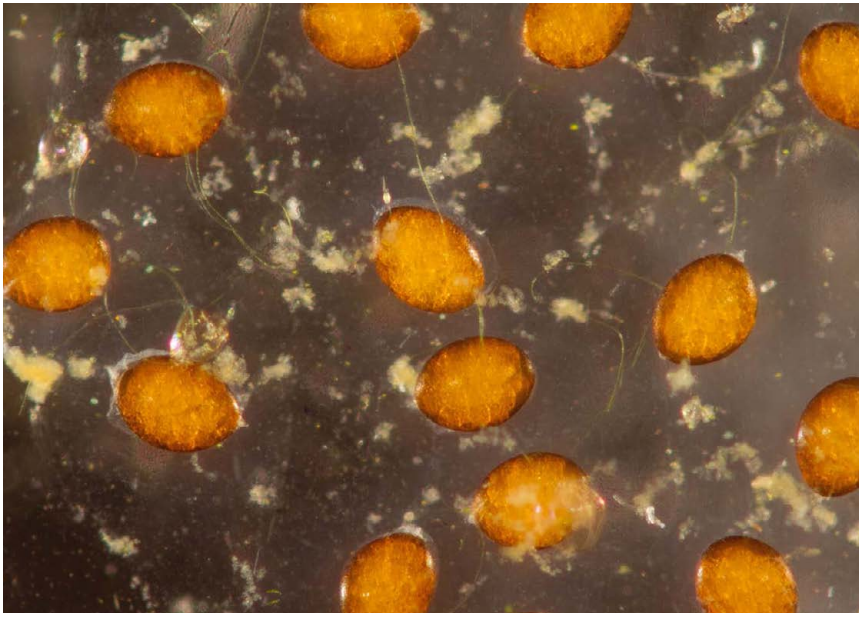


Plate 4. Eggs showing internal yolk globules and surrounded by gel.

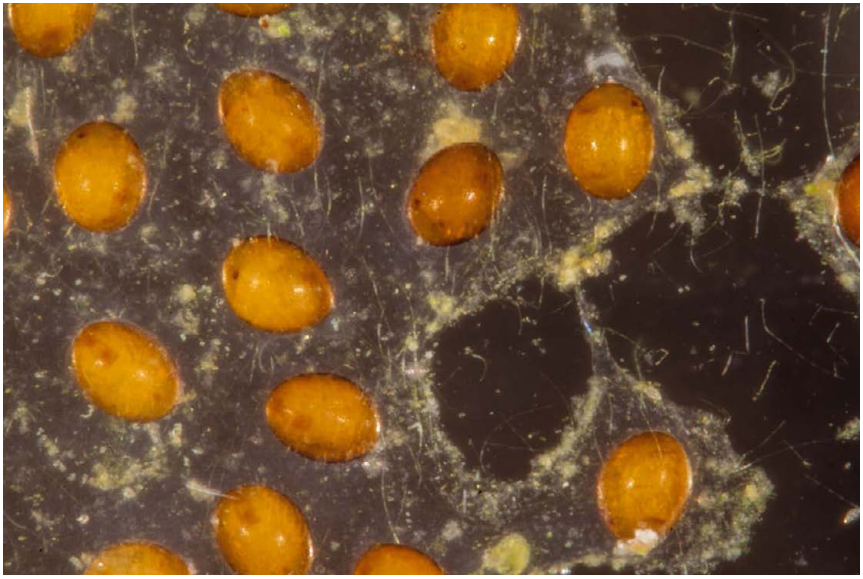


Plate 5. 9 days after oviposition. The eggs are showing early stages of internal differentiation with signs of pigmentation appearing. The margins and extent of the gel around the eggs are clearly visible.

and attracted small copepods in the pond water to feed on it. All other eggs were ignored by them and remained intact.

Nine days after oviposition (11 September 2017) most of the eggs showed signs of development and internal differentiation (Plate 5). Development of the legs could be observed with other darker pigmented areas that would develop into the eyes and mandibles. A dark patch also developed at the opposite end of the egg. Red-eye pigmentation had become very distinct by 13 September 2017, along with other darker areas (Plate 6). Eye spots, which are formed from pigmented ommatidia, become very evident during development. Here they started to appear after 7-9 days, being very discernible by 9-11 days. At this stage eggs that did not have clearly visible eye spots did not develop any further (n=11).

Fifteen days (17 September 2017) after oviposition hatching of eggs commenced and many small stadium 2 larvae were present in the water (Plate 7). Newly hatched larvae were first observed at 07.45, hatching having presumably started during early daylight hours.

Whilst the high number of eggs and observation technique did not facilitate the detailed timetable of hatching to be recorded it was noted that the eggs at the periphery of the egg clusters hatched earlier than ones in the middle of the egg clusters. Those in the middle remained unhatched during peak periods when most hatching was observed (Plate 8).

By 21 September 2017 (19 days after oviposition and 4 days after hatching started) most of the eggs had hatched. The embryos in the remaining eggs showed occasional movement. In addition to some rotational movement of the embryo the pigmented mandibles were observed to move occasionally. On 23 September 2017 two more eggs hatched followed by the final three on 24 September 2017, hence hatching occurred between 15 and 22 days after oviposition. On this date 11 eggs (2.46% of those fertilised) remained unhatched and stayed this way. Each of these still showed globules of yolk but did not exhibit the development of any pigmentation or visible structure. The reasons for lack of development in these eggs are unknown.

By the time of hatching the head typically appeared ventral side uppermost (Plate 9). The hatching process started with the dorsal surface of the head appearing first and presumably initiating the split (Plate 10). Initial splitting of the egg was rapid and difficult to observe and record. The times recorded for the hatching process therefore began just after the egg shell had split and the larva was emerging. The dorsal surface of the head of emerging larvae had a thin, red pigmented line that formed a crest (Plate 10) which disappeared as the hatching

Plate 6. 11 days after oviposition. Pigmented eye spots (e) and legs (l) can be seen clearly.

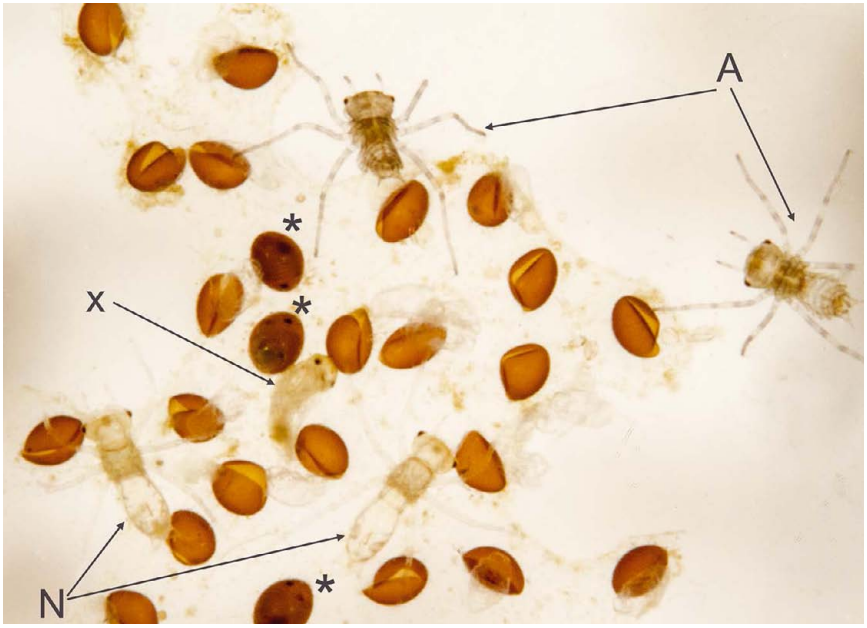


Plate 7. 15 days after oviposition, showing unhatched eggs (*), hatched eggs, larvae emerging from eggs (x), newly emerged stadium 2 larvae (N) and more advanced stadium 2 larvae (A).

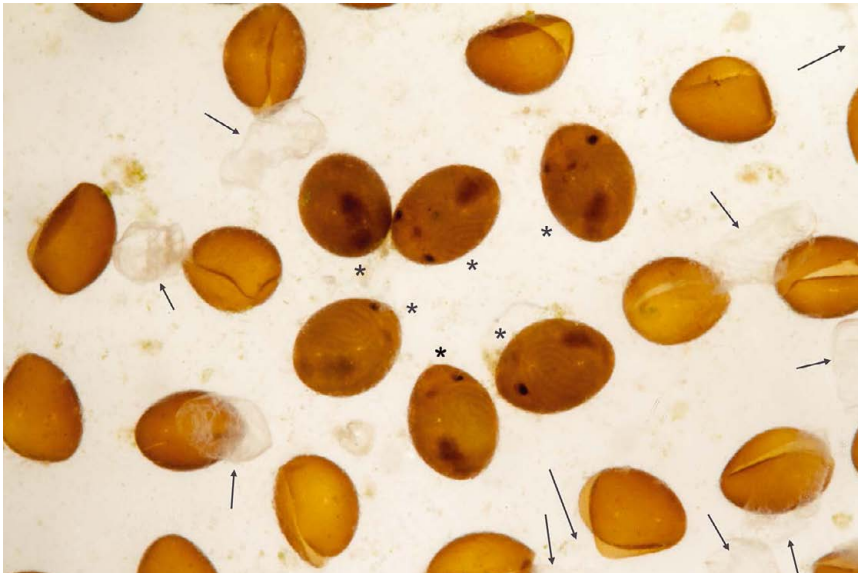


Plate 8. 15 days after oviposition showing six darker, unhatched eggs (*) in the middle of a cluster of hatched eggs. These were the last six eggs to hatch. The hatched eggs show the prolarval exuviae still attached (arrowed).

progressed and was not visible on stadium 2 larvae. This may play a role in initiating the splitting of the egg. At the initial stage of hatching it was difficult to differentiate between the head of the emerging stadium 2 larva and the prolarva but it was apparent that the stadium 2 larva emerged from the prolarval exuvia as the hatching process progressed. In all observed hatchings the prolarval exuvia remained attached to the vacated egg shell after the stadium 2 larva was free (Plates 11, 12). In larvae that emerged vertically and therefore free of the gel the time taken for the hatching process from the first sign of the egg shell splitting to the stadium 2 larva being free of the prolarval exuvia was between 16-22 minutes.

The direction of emergence varied. Whilst most larvae emerged vertically (Plate 13A,B) others appeared to emerge laterally and then wriggle through the mass of gel before freeing themselves (Plate 14). The time taken for these larvae varied depending on their ability to free themselves from the gel around the eggs. This took longer than larvae emerging vertically and for some individuals the process took nearly an hour.

Once free of the egg and prolarval exuviae the stadium 2 larvae quickly became active. Initially the abdominal contents of the larvae appeared clear (Plate 15) with much of the thorax appearing darker with yolk reserves. Following a series

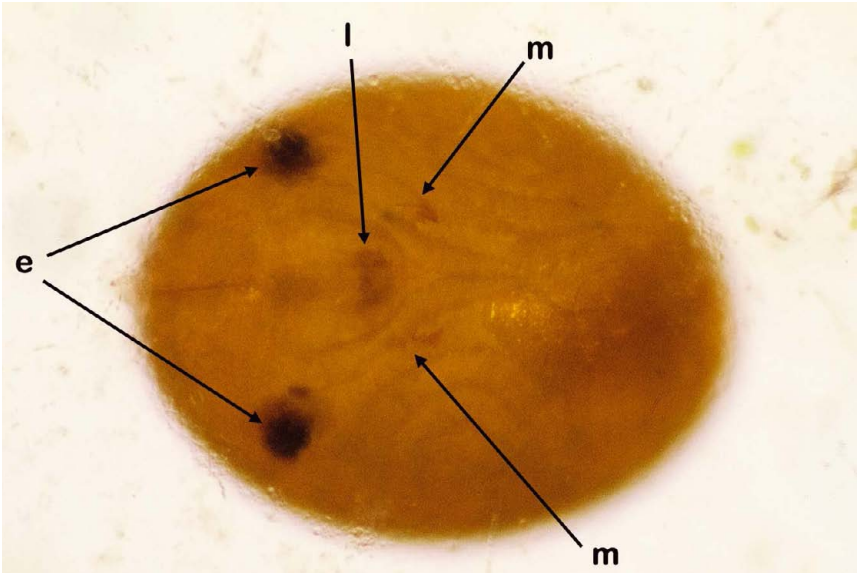


Plate 9. An egg showing the venter up position of the embryo. The dark pigmented eye spots (e), labrum (l) and tips of the mandibles/maxillae (m) can be seen.

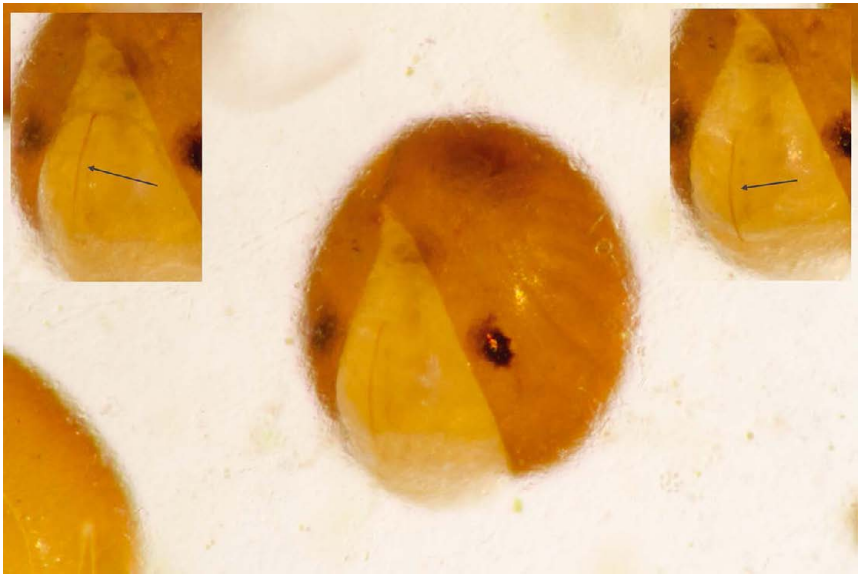
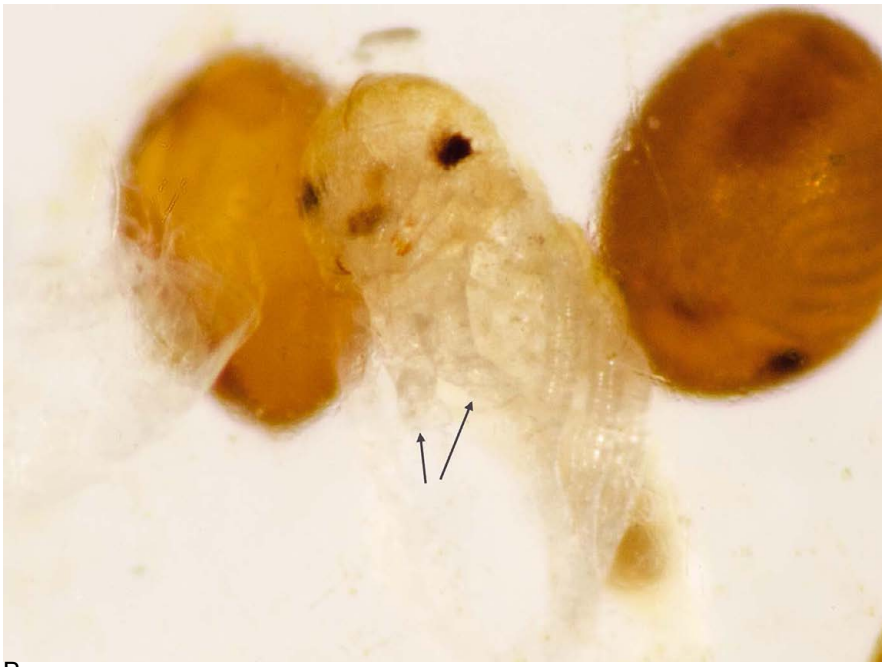


Plate 10. The hatching process, which is initiated by the top of the head pushing through the split in the egg shell. The insets show the red pigmented line (arrowed) that may play some role in initiating the splitting of the egg shell.

Plate 11. A newly emerged stadium 2 larva adjacent to its prolarval (stadium 1) exuvia (arrowed), which is still attached to the egg.

Plate 12. A prolarval exuvia attached to the egg.

A



B

Plate 13. Larvae emerging in a vertical posture. A) Two larvae emerging, clear of the egg and surrounding gel. B) A larva emerging, showing two structures below the head (arrowed) which are the two sides of the labium before it covers the front of the head.

Plate 14. A larva that has emerged laterally and is trying to free itself from the gel around the eggs. The transition to a fully active stadium 2 larva takes longer as the larva struggles to free itself from the gel.

Plate 15. A newly freed larva showing its relatively clear body. The yolk globules stored in the thorax are visible (arrowed).

Plate 16. A larva over an hour after hatching, showing darkening of its abdomen.

Plate 17. Two stadium 2 larvae sparring using their legs.

Plate 18. Two stadium 2 larvae showing aggression and spacing out using their forelegs. (scale bar = 1.0mm).

Plate 19. The stadium 2 larva on the right has just reacted to movement by rapidly striking with its mask which can be seen partially expanded.

of abdominal pumping movements presumably to activate the respiratory system, the body contents darkened and became more evident (Plate 16). During the entire hatching process all larvae except one successfully completed the transition to the stadium 2 stage. That larva separated from the egg and prolarval exuvia but struggled to separate itself from the gel. After several hours its attempts to free itself diminished and it showed no further signs of life. The following day it was presumed to be dead and subsequently decomposed.

As the larvae became more active they came into contact with other larvae that had hatched in close proximity. Interactions resulted in some aggression between individuals. Larvae were observed to use their legs to fend off other larvae in close proximity (Plates 17,18). This serves as an effective mechanism to facilitate spacing out and dispersal of the larval cohort.

It has been suggested that larvae can become cannibalistic in the absence of a suitable food supply. In this study no observations of cannibalism were observed, although some larvae would strike out with their labium when a sudden movement occurred nearby (Plate 19). Inter-larval aggression mainly comprised movements of the legs and typically resulted in one of the larvae moving away. Stadium 2 larvae had yolk reserves which were visible for several days. However, to avoid the risk of starvation since none of the provided prey items were seen to be taken, the stadium 2 larvae were released back into the garden pond after seven days observation. It is more likely that intra-species predation occurs when larvae of later stadia are also present in the same area.

Discussion

Exophytic eggs of many odonate species, including *Sympetrum striolatum,* have a thick envelope comprising several layers, the major part of which is the egg shell. The outer layer or exochorion transforms into the gel layer which expands on contact with water. In some species this is reported to be sticky (Corbet, 1999) and the observations reported here suggest that the outer layer of the eggs of *S.striolatum* is initially sticky, enabling it to adhere to the substrate.

Duration of the egg stage depends largely on temperature (Corbet, 1999) and one assumes that under the controlled and relatively stable temperature conditions in this study that hatching would take place in less time than in the natural conditions of the pond. The eggs hatched between 15 and 22 days after oviposition at a stable temperature of 19.7°C, which did not vary over the time between laying and the eggs hatching. In contrast, the temperature measured in the garden pond varied significantly during the day when the pond was in full sun (up to 21.5°C) and overnight (17.5°C). The differences between

hatching times in controlled and natural conditions was not investigated. Eggs of *Cordulia aenea* Downy Emerald observed under similar controlled conditions hatched after 18 days (Cham, 2004).

Prior to hatching and the rupturing of the egg shell, various movements of the embryo could be observed. In some cases these included movement of the mandibles which are considered to be swallowing movements (Corbet, 1999).

It has been reported that the reduced access to oxygen experienced by the innermost eggs in a clump of *Antipodochlora braueri* is likely to cause slightly delayed hatching (Winstanley in Rowe, 1987). *Sympetrum striolatum* eggs in this study settled in a monolayer on the bottom of the glass dish and were adequately covered by water throughout. Eggs in the middle of clusters were the last to hatch suggesting that there may be another stimulus for hatching. Delayed hatching eggs appeared to have similar features to the other eggs at the point of hatching. The movement of the dish during observations under the microscope agitated the water sufficiently to avoid any dissolved oxygen gradients. Therefore reduced oxygen levels alone would not adequately explain the delayed hatching of the innermost eggs in these clumps. Some other stimulus appears to be in operation and this remains an interesting subject for further study.

Corbet (1999) suggested that yolk granules impart the dominant colour to an egg. This may partly be the case for eggs of *S. striolatum* but it is not the major contributor to the overall colour. On laying, eggs have a transparent chorion with an even distribution of yolk which gives them a homogenous appearance and white colouration. Darkening of the fertilised egg is mainly due to tanning of the chorion which occurs soon after oviposition. Immediately after hatching the egg shells appeared slightly lighter but still retained their dark brown colouration.

Corbet (1999) referred to gravity dependent rotation of the developing embryo during the latter stages of odonate egg development whereby they adopt a venter up position (Plate 9). This means the emerging larva can hatch away from the substrate, in this case the gel mass. Whilst rotation of the embryos was observed and many embryos adopted a venter up position there did not appear to be a consistent pattern to it. During the hatching process most larvae were observed hatching and emerging in a vertical posture. These larvae quickly and relatively easily separated from the gel and the stadium 1 (prolarval) exuvia. A relatively high number of larvae also hatched and emerged laterally. These larvae would find themselves engulfed in gel and had to struggle to free themselves. Of the eggs that hatched there was one larval fatality, assumed to be due to its inability to free itself from the gel and using up all its available energy.

In the present study, fertility was observed to be close to 100% (99.55%) with only two unfertilised eggs observed. Of the fertilised eggs 11 (2.46%) failed to develop to the point where differentiation could be observed and one stadium 2 larva failed to free itself from the gel. Fertility values of 63-88% have been recorded for *S. striolatum* in southern Spain (Testard, 1972) but these were regarded by Corbet (1999) to be unusually low because the observations were made in December when the temperatures may have reduced copulation efficiency.

There has been discussion (Corbet, 1999) concerning whether the prolarval stage should be considered to be the 1st stadium larva. To adopt Corbet's preferred notation (Corbet, 1999) the larva that separates from the egg and prolarval exuvia is considered to be the stadium 2 larva. From the observations made during this study the prolarval exuvia always remained attached to the egg (Plates 8, 18, 19). For species of Odonata that oviposit endophytically out of water a free prolarval stage is an important adaptation for the larval stage to reach water (Corbet & Brooks, 2008). For exophytic species, such as *S. striolatum*, which oviposit directly into water, the prolarval stage lasts no more than a few minutes as the larva exits the egg. Whilst referred to as the stadium 1 larva the prolarva is unlike the following stadia, forming a sheath around the emerging stadium 2 larva. Here the prolarval exuvia appears to serve an initial function to 'smooth the way' for the larva leaving the egg. In all observations, the stadium 2 larva was already emerging from the prolarval exuvia during the hatching process. The stadium 1 prolarval stage therefore effectively overlaps with the emerging stadium 2 larval stage.

Immediately after the larva has separated from the prolarval exuvia and fully expanded its legs it has relatively clear body contents. At this point these larvae were observed making pumping movements of their abdomen as the respiratory system becomes functional (Corbet, 1999). The body contents darken within one to two hours, by which time the larvae become more active. Stadium 2 larvae have distinctly banded legs that are covered in fine hairs.

Conclusions

The egg and early larval stage of British dragonfly species is the least-studied but is crucial to the subsequent development and survivorship of a species. The eggs of species that oviposit exophytically are very difficult to locate in situ and require the capture of an ovipositing female to collect eggs. The gel formed from the exochorion adheres the eggs to the substrate and also provides a protective envelope for the developing eggs, potentially protecting them from predation and bacterial and fungal infection. The unhatched eggs were observed under

the microscope for 42 days after oviposition and only 2% showed any signs of decomposition or fungal growth, suggesting that the gel is indeed an effective barrier. Fertility was 99.55% and survival rate from oviposition to free-swimming stadium 2 larvae was 96.88%. The eggs observed in this study developed directly after oviposition. However, it is known that *Sympetrum striolatum* eggs laid late in the season go into a facultative diapause (Corbet & Brooks, 2008; Miller, 2014). It would be interesting to assess the latest dates when this occurs and if climatic changes influence this in the future. There are many opportunities for further observations and studies of the egg and early larval stages and it is hoped that others will be encouraged to undertake further work.

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A key for separating early larval instars of four common co-occurring damselfly species in the U.K.: *Ischnura elegans* (Vander Linden), *Erythromma najas* (Hansemann), *Coenagrion pulchellum* (Vander Linden) and *Enallagma cyathigerum* (Charpentier)

Ora E. Johannsson

Department of Zoology, University of British Columbia, Vancouver, B.C., Canada, V7R 4A5

Summary

Ecological studies of species assemblages require the identification of all individuals. This becomes difficult in aquatic insect assemblages where traites change continuously throughout development and separation of similar species is difficult, especially in earlier instars. A key was developed and is presented here to separate the majority of instars of a common assemblage of four damselfly species (*Ischnura elegans* (Blue-tailed Damselfly), *Erythromma najas* (Red-eyed Damselfly), *Coenagrion pulchellum* (Variable Damselfly) and *Enallagma cyatherigum* (Common Blue Damselfly)) in the U.K.. This was part of a study on the factors leading to their co-existence.

Introduction

Biologists have long been interested in animal communities and the factors which govern their structure. The concept of n-dimensional niche space (Hutchinson, 1957) and the ideas presented by Hutchinson (1959) in his paper asking 'Why are there so many kinds of animals?', fuelled more effort into research on the factors governing the co-existence of similar species to this day. This knowledge increases our understanding of how biodiversity is maintained and the factors governing it.

Similar species which co-occur, divide their use of habitat resources along three axes: time, space, and food (Pianka, 1973; Schoener, 1974). This separation decreases competition, permits reproductive isolation, and allows each population access to sufficient resources to support itself. The importance in understanding how animals utilize and share habitat resources, rests in the need to protect sufficient diversity within a habitat to support entire assemblages, maintaining biodiversity. Johannsson (1978) showed that spatial, and to a

lesser extent temporal, separation allowed for the co-occurrence of a damselfly assemblage common to the British Isles.

The damselflies *Ischnura elegans* (Blue-tailed Damselfly), *Erythromma najas* (Red-eyed Damselfly), *Coenagrion pulchellum* (Variable Damselfly) and *Enallagma cyatherigum* (Common Blue Damselfly) often coexist, sharing the aquatic habitat. *Ischnura elegans* and *E. cyatherigum* are ubiquitous species found throughout England, Scotland and Ireland (Corbet *et al*., 1960; Hickling *et al*., 2005; British Dragonfly Society, 2017). *Coenagrion pulchellum* is found throughtout most of Ireland, while in Britain, pockets of abundance occur in the east below latitude 53°N, as well as in Dumfriesshire in the north (Cham *et al*., 2014). *Erythromma najas* was considered a southern species found principally in the south, middle and eastern regions of England (Corbett *et al*., 1960; Hickling *et al*., 2005; British Dragonfly Society, 2017.). However, in recent years it has moved northward and can now also be found in Cheshire and southern Yorkshire (Cham *et al*., 2014). These four species inhabit a wide range of freshwater habitats (Corbet *et al*., 1960; Cham *et al*., 2014; British Dragonfly Society, 2017): *Enallagma cyathigerum* is common in most waters; *I. elegans* occurs in a variety of lowland habitats including slightly saline or polluted waters; *E. najas* inhabits lakes, ponds, and slow running water; while *C. pulchellum* can be found in coastal marshes, fens, dykes and standing water associated with good vegetation. Thus, there is much overlap in the geographical distribution of these species in the British Isles. Indeed, the overlap is increasing as the distributions of the more southern species are gradually extending northward, presumably as a result of the increasing temperatures associated with climate change (Corbett *et al*., 1960; Hickling *et al*., 2005; Brooks *et al*., 2007; Mill *et al*., 2010).

Current keys to larval identification are generally restricted to characteristics of the final larval instar (e.g. Gardner, 1954) or the final larval instar and exuvia (Cham. 2009). However, in order to study the ecology of larval odonates, keys need to be developed to separate species at early instars. The key presented here was developed during a study of the co-existence of the above assemblage of damselflies in the Norfolk Broads (Johannsson, 1976, 1978), which are shallow, water-filled peat diggings originating from the Middle Ages found within the valleys of the rivers Bure, Thurne, Ant and Yare east of Norwich (Ellis, 1965).

Material and Methods

Damselfly larvae were collected from two, small land-locked Broads, Upton and Alderfen, from September 1972 until April 1974. The two Broads had a similar substrate structure: a shallow, reed-covered, hard peat layer, partially

surrounding an open basin with a liquid mud substrate. A short slope of reedless, hard peat joined the two regions. Both Broads supported nearshore waterlily beds. In Upton Broad, macrophytes (principally *Najas marina*, the Holly-leaved Naiad) were abundant on the mud from spring to autumn. Alderfen Broad had lost the majority of its macrophyes as nutrient concentrations in the water increased (Phillips *et al*.,1978).

Samples were collected from all three habitats at six week intervals in order to follow the movement of larvae amongst habitats through the year and to develop a holistic picture of their life histories. Artificial macrophytes as designed by Whortley (1974), a modification of those of Macan and Kitching (1972), were deployed in the reedbeds. Each was constructed from 50 strands of bouyant, polypropylene rope attached to a plastic mesh base (20 cm x 20 cm) and secured in position with a long bamboo pole. They were left in position in the reedbed for six weeks before they were retrieved. A vacuum system worked well in sampling the intermediate zone. Each $1m^2$ area was vacuumed for six minutes per sample. Mark and recapture studies found that 36% of the damselfly larvae were caught in the first sampling (Johannsson, 1976). The soft mud was sampled with shallow hoop nets in order to capture the larvae living on the surface of the mud. The hoops were lightly covered with mud and left for six weeks before being retrieved and set again. For more detailed descriptions of sampling methods and associated tests, please consult Johannsson (1976, 1978).

Upon retrieval, the artificial macrophytes and the vacuum samples were bagged individuallly. The mud samples were sieved (#30 sieve, 595 microns) and then bagged. The samples were stored in a cold room (≤5^{o}C), until they could be processed. Each sample was washed into a white tray and the damselflies removed. The larvae were identified to species using Gardener's key (Gardner,1954) which is based on characteristics of the labium (lower mouth part) of the ultimate instar (Fig. 1). Those larvae which could not be identified, were kept in individual plastic cups filled with water brought back from Upton or Alderfen Broad. The water level was maintained by adding distilled water when necessary. The larvae were fed hatched *Artemia salina* twice a week, augmented with any *Daphnia* and chironomids which came into the laboratory in samples. The pots were kept at room temperature, approximately 18^{o}C - 20^{o}C.

For each individual, the exuvia from each larval moult were kept together in a labelled vial containing 70% alcohol. When the larva reached an instar where it could be identified or had died, its mouth parts and those of its exuviae were mounted in polyvinyl lactophenyl on a microscope slide and covered with a coverslip. The labia were then examined under a compound microscope.

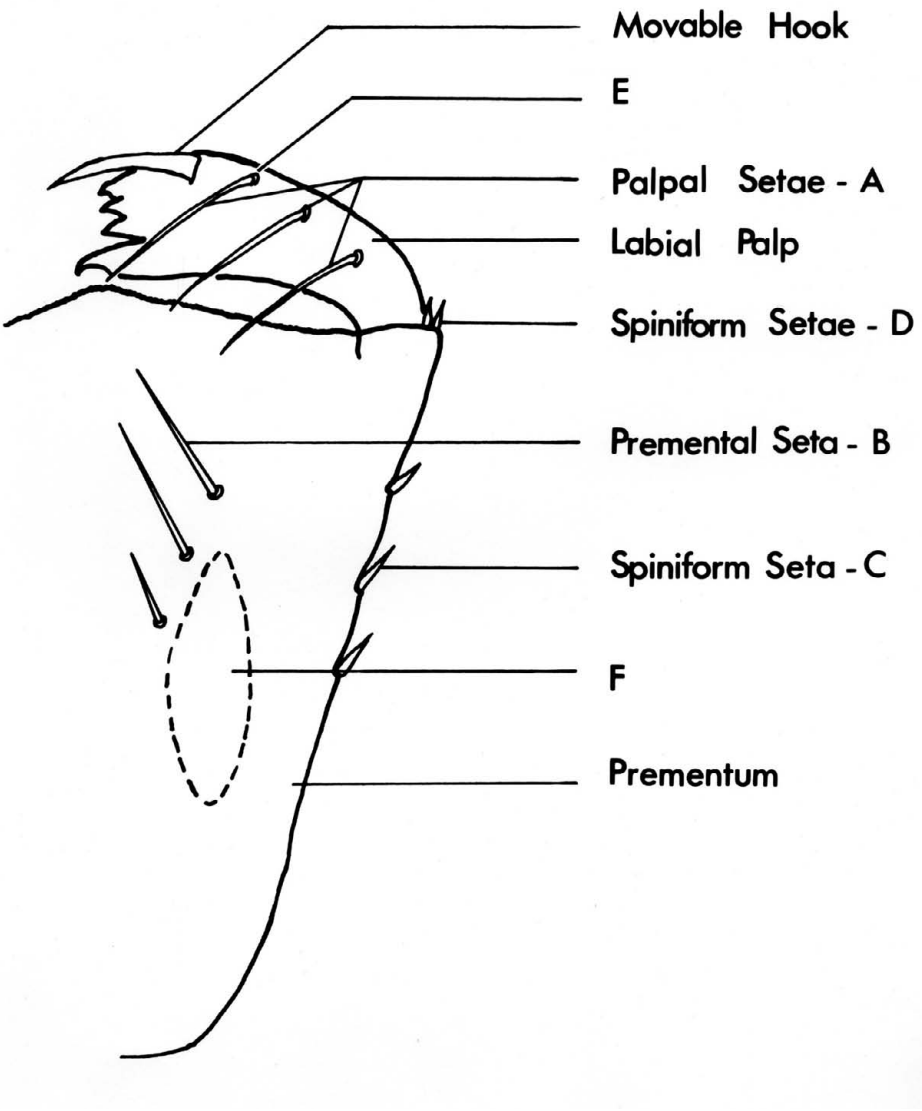


Figure 1. The right half of a zygopteran labium. A, palpal setae; B, premental setae; C, spiniform setae on the sides of the prementum; D, spiniform setae at the articulation between the prementum and its palps; E, the position of the long spine, present in *Enallagma cyathigerum*, at the base of the distal seta on the labial palp (Fig. 2); F, the position of the array of short spines characteristic of later instars of *Ischnura elegans* and *Enallagma cyatherigum*. Characters A - F are used in the key to separate immature larvae in the assemblage *Ischnura elegans*, *Erythromma najas*, *Enallagma cyathigerum* and *Coenagrion pulchellum.*

This enabled changes in labial characteristics to be charted throughout the development of individuals and species. Based on these developmental timelines, a key was constructed to separate the species at different instars.

The labium of a damselfly consists of a prementum and a pair of labial palps (Fig. 1). The prementum can develop setae near the mid-line (premental setae) and along its lateral edge (spiniform setae). Short spines may appear during development within a field behind and between the setae and the lateral edge. Spiniform setae also occur at the base of each labial palp where it articulates with the prementum. Each labial palp has a thickened, mobile spine (hook) near its outer tip and may have palpal setae along its outer edge (Fig. 1). These features change with development. Therefore, the following were noted for each exuvia:

A. the number of setae on each labial palp
B. the number of premental setae on each side
C. the number of spiniform setae on each lateral edge of the prementum,
D. the number of spiniform setae on the prementum at the articulation with each labial palp.
E. The presence or absence of a large thick-bodied spine near the base of the most distal seta of the labial palp
F. The presence or absence of small spines proximal and lateral to the premental setae in the area labelled 'F' (Fig. 1)

Results

Damselflies undergo 9 to 13 moults (Corbett *et al*. 1960). The spines and setae of the prementum and labial palps appear gradually throughout development so that small changes occur from one moult to the next. One can observe different numbers of lateral spiniform spines on the two sides of the prementum; also premental seta which may only be partially developed and hence recorded as half a seta. The number of labial setae do not increase with each moult but remain constant over several moults. This leads to variability in the other setal and spine characteristics observed for a given number of setae on the labial palp. These developmental factors were used to construct the key (Table 1).

Enallagma cytherigum can be separated from the other three species by the presence of a distinctive spine near the base of the distal seta on each labial palp (Plate 1), which is present even in very small instars where A:B:C:D = 1:0:0:0 (Fig. 1). (A:B:C:D refers to characteristics of the setae and spines on the prementum and labial palps that were used to develop the key). The presence of only one seta on the labial palp indicates that these were first instar larvae

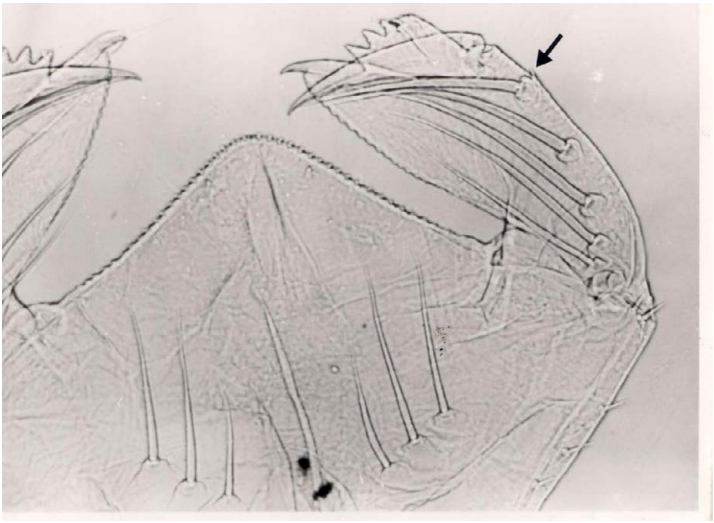
Table 1. A key to the identification of early and late instars of the larvae of the damselfly assemblage *Ischnura elegans, Erythromma najas, Enallagma cyatherigum* and *Coenagrion pulchellum,* based on characteristics of the labium. Ratio A:B:C:D refers to the following attributes of the labium (Figs 1, 2).

A: The number of setae on each labial palp
B: The number of premental setae on each side
C: The number of spiniform setae on each lateral edge of the prementum
D: The number of spiniform setae on the prementum at the articulation with each labial palp

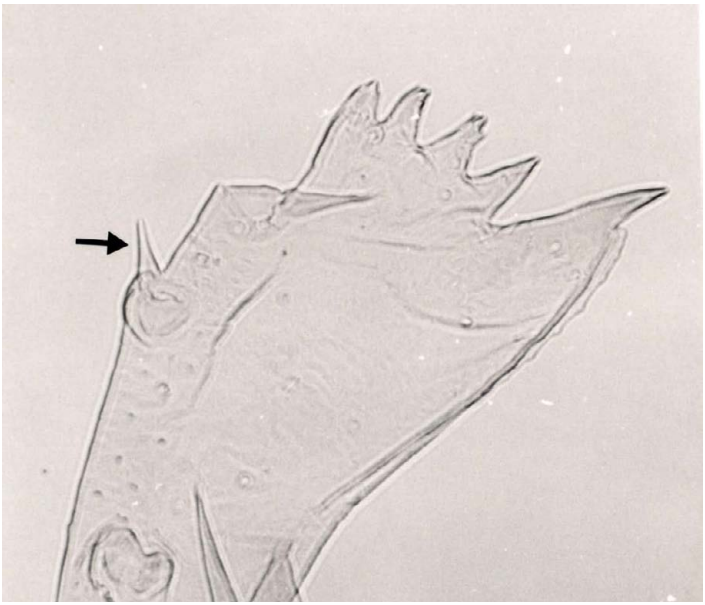
Other characters used are:
E: The presence or absence of a spine at the base of the distal seta on the labial palp
F: The presence or absence of short spines proximal and lateral to the premental seta

			Characteristic	Identification/Instruction
1			Large spine at the base of the distal seta of the labial palp	*E. cyatherigum*
			No spine present (Fig. 2)	2
2a			6 setae on the labial palp	
	3		2 lateral spiniform setae	*C. pulchellum*
			>2 lateral spiniform setae	4
	4		3 spiniform setae at the articulation* and ≤4 premental seta	*E. najas*
			≥3 spiniform setae at the articulation and >4 premental seta	*I. elegans*
2b			5 setae on the labial palp	
	5		3 premental setae	6
			4 premental setae	8
	6		1-2 lateral spiniform setae	*C. pulchellum*
			>2 lateral spiniform setae	7
	7		Short spines proximal and lateral to premental setae	*I. elegans*
			No such spines present	*E. najas*
	8		2-3 lateral spiniform setae	*C. pulchellum*
			5-8 lateral spiniform setae	*I. elegans*
2c			4 setae on the labial palp	
	9		2 premental setae	10
			3 premental setae	11
	10		Characteristics C:D = 2-3:≥2	*I. elegans*
			Characteristics C:D ≠ 2-3:≥2	*E. najas* & *C. pulchellum* indistinguishable
	11		1 lateral spiniform seta	*E. najas* & *C. pulchellum* indistinguishable
			>1 lateral spiniform setae	12
	12		3 spiniform setae at articulation	*I. elegans*
			1-2 spiniform setae at articulation	13
	13		Ratio of measures A:B:C:D ≠ 4:3:4:2	*E. najas*
			Ratio of measures A:B:C:D = 4:3:4:2	14
	14		Short spines or 1-2 spatulate spines proximal and lateral to premental setae	*I. elegans*
			No such spines present	*E. najas*
2d			3 setae on the labial palp	
	15		No lateral spiniform setae	*C. pulchellum*
			Lateral spiniform seta(e) present	16
	16		2-3 spiniform setae at articulation	*I. elegans*
			<2 spiniform setae at articulation	*E. najas*
2e			1-2 setae on the labial palp	*I. elegans, E. najs* & *C. pulchellum* indistinguishable

*articulation refers to the articulation of the labial palp with the prementum



A



B

Plate 1. The labial palps of early instar larvae of (A) *Ischnura elegans* and (B) *Enallagma cyatherigum.* The arrows indicate the base of the distal labial palp seta. Note the absence of a long spine in this position in *I. elegans* and its presence in *E. cyathigerum*. This long spine is not to be confused with the very short spine that can just be seen at the base of the distal seta in *I. elegans*.

(Corbet *et al.*, 1960). Care is needed when using this character because the earlier instars of *Ischnura elegans* and *Erythromma najas* have small, scale-like spines in the same position at the base of the distal seta on the labial palp. (Fig. 2).

The number of setae on the labial palp is the first character to consider as the relative number of other setae and spine types change among the species at each addition of a seta to the labial palp. The species separate easily at 5 and 6 labial setae. The key construction becomes more complex at 4 labial setae, and at certain combinations of setae (4:2:x:x and 4:3:1:x) not all *E. najas* can be separated from *Coenagrion puchellum.* Separation of the species is possible at 3 setae on the labial palp; however, at 1 or 2 labial setae, only *E. cyatherigum* can be confidently identified – by the spine at the base of the distal labial seta (see above) (Plate 1).

Ischnura elegans grow spines proximal to the premental setae and further toward the edge of the prementum. These spines first appear at a moult in the stage when the labial palp has three setae. The number of spines increases through development and extends the region of spines more distally. In *E. cyatherigum,* two or three spines develop far back on the prementum in the last instars. If the labial palp is absent, this difference in spine development may help to separate these two species.

In order to aid with the use of the key, examples of the premental and labial palp characteristics of actual larvae are provided for each increment in setae on the labial palp (Table 2). Exploring these examples will provide a better feel for using the key.

Discussion

The key presented in the current study uses the number and arrangment of setae and spines on the prementum and labial palps to distinguish between the larvae of *Ischnura elegans*, *Erythromma najas*, *Coenagrion pulchellum*, and *Enallagma cyatherigum* at most larval stages, four species that co-occur in Upton and Alderfen Broads. *Enallagma cyathigerum* can be identified as of the first instar; the other species can be separated when the labial palp has three or more setae, except for a portion of the *E. najas* and *C. pulchellum* with 4 setae on the labial palp. In *C. pulchellum*, the third seta appears in the 4th instar (Balfour-Browne, 1909). Too few individuals of the different species were collected to try to separate them at the earliest instars.

Gardener (1954) and Cham (2009) have also used characteristics of the

Table 2. Examples of the labial characteristics of a range of larval instars of *Ischnura elegans, Erythromma najas, Enallagma cyatherigum* and *Coenagrion pulchellum*. The species can be identified using these characteristics (Table 1). The data are from individuals collected at Upton or Alderfen Broad between 1972-1974.

Species	*Enallagma* spine (Fig. 2)	Spines in Area 'F' of Fig. 1	No. of palpal setae	No. of central prementum setae	No. of lateral premental setae	No. of setae at articulation of labial palp
Ischnura	0	0	3	1½	1	2
elegans	0	0	3	2	1	2
	0	1	3	2½	3	1½-3
	0	1	4	2	2	2-2½
	0	1	4	2	3	3
	0	1	4	3	3-5	3
	0	1	4	2½	2-3	2-3
	0	1	4-5	3-4	6-7	2½-3
	0	1	5	4	5-7	2½
	0	1	5	3½	4	4
	0	1	5-6	4	8	3½
	0	1	6	3½	8-9	3-4
	0	1	6	4½	8	4
Erythromma	0	0	3	2	1	1
najas	0	0	3	2	1	1½
	0	0	3	2	1-2	1
	0	0	3	2-3	1	1½
	0	0	3	2½	1	1
	0	0	4	3	2-3	1½-2
	0	0	5	3	3	2½
	0	0	4-5	3½	3-4	2
	0	0	5	4	3	1-3
	0	0	5	3-3½	2-4	2
Coenagrion	0	0	3	1½	0	1
pulchellum	0	0	3	2	0	1
	0	0	3	2	0	2
	0	0	4	3	2-3	2
	0	0	5	3	2	2
	0	0	5	3½	3	2½
	0	0	5	3½	1	3
	0	0	5	3-4	2-3	2
	0	0	5	3½-4	3	3
	0	0	5	4	2	3
	0	0	6	4	2	2½
C. pulchellum	0	0	4	2	1	2
or E. najas	0	0	4	2	1	1-1½
	0	0	4	2½	1	2
	0	0	4	3	1	1
	0	0	4	3	1	1½
	0	0	4	3	1	2
Enallagma	1	0	3	1	1	1
cyathigerum	1	0	3	2	1	1
	1	0	3	2	1-2	1
	1	0	3	2½	3	1-2
	1	0	4	2-3	3	1-1½
	1	0	4	2½-3	4	2
	1	0	4	2½-3	4-5	2
	1	0	4½	3-3½	5	2
	1	0	5	2½	4-5	2
	1	0	5	2½-3	6-8	2½
	1	0	5	3	5-6	2-3
	1	0	5	3	8	2-2½
	1	0*	6	3	6-8	2-2½
	1	0*	6	3½	8-10	2-2½
	1	0*	6	3½	10	3
	1	0*	6	4	8	2

*may have a few spines far back

prementum and labial palps to separate damselfly species, although only in the ultimate instar. Gardner (1954) used the short spine near the base of the distal seta of the labial palp to distinguish *E. cyathigerum*, and the premental and labial setae to separate some of the species of *Coenagrion*. Cham (2009) used the angle between the premental setae on either side to distinguish beween *C. pulchellum* and *Coenagrion puella* in late instar larvae and exuviae. Otherwise, the use of premental and labial palp characteristics has been limited in taxonomic keys because the number of spines and setae can be variable (Gardner, 1954). Variation in number could be related to the number of moults undergone before the larvae reach the ultimate instar. With knowledge of the variation in setae and spine numbers, some of this variability can be accounted for, allowing the labium to be of greater taxonomic value. The present key works because it considers a combination of setae and spines within the groupings created by the number of setae on the labial palps.

Chowdhury & Corbet (1987) also developed a key to separate earlier instars of *I. elegans* and *E. cyathigerum* down to a body length of 7.6 mm (excluding antennae and caudal lamellae) based on external morphology, avoiding damage to the larvae associated with examination of the labium under the microscope. The present study found that in *E. cyathigerum* the large spine at the base of the distal seta of the labial palp is present even in the first instar. Therefore, in situations where other species (*E. najas* and *C. pulchellum*) are present, or when the larvae are preserved on sampling and/or are very small, the key presented here provides a valuable option in separating these two species. Chowdhury and Corbet's method (Chowdhury & Corbet, 1987) should be tested on the earliest instars to see if it might be used to separate *I. elegans* from *E. najas* and/or *C. pulchellum* in the first three instars.

We do not know how selective pressures within individual ecosystems may influence the characteristics of the prementum and labial palps. Further development of the key should include testing its ability to separate these four species at other sites. It is also hoped that the key can be expanded to include the larvae of other zygopteran species which might co-occur with the above four species.

In summary, the key is able to separate the majority of early larvae of *I. elegans*, *E. najas*, *C. pulchellum*, and *E. cyatherigum*, providing a means of rapid identification of most larvae and reducing the need to breed any but the smallest larvae for identification.

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The role of the abdomen of anisopteran larvae in respiration, locomotion and prey capture: A review – 2: Mechanics and neural control.

Peter J. Mill

School of Biology, University of Leeds, Leeds, LS2 9JT

Abstract

The dorso-ventral movements of the floor of the larval abdomen produce pressure changes in the body cavity that are used for ventilation of the gills in the branchial chamber, for jet-propulsive swimming and for labial mask extension (prey capture). The various mechanisms and their neural control are reviewed. The techniques employed for the mechanical and physiological studies are outlined. The review is preceded by a description of the types of nerve cell (neuron) and how they convey information. Details of the mechanics and physiology have been obtained mostly from aeshnid larvae except in the case of labial mask extension.

Introduction

In part 1 of this review (Mill, 2016), the structure of the abdomen, including both the nervous and muscular systems, was described; also a description was given of the muscles controlling the labial mask and the labial palps. In this part of the review, the mechanics and neural control of respiration, jet-propulsive swimming and labial mask extension will be outlined. Most of the information on respiration and jet-propusion comes from studies on aeshnid larvae, while that on labial mask extension is from both aeshnids and libellulids.

Ventilation, jet-propulsive swimming and labial mask extension all involve an increase in internal body pressure brought about by lifting of the floor of the abdomen (sterna). In all three this causes compression of the branchial chamber. The branchial chamber contains gills and, according to Tillyard (1916), its respiratory rôle was first noted by Poupart (1702). Ventilation involves water being ejected through the anus (expiration) and sucked back in (inspiration). The rapid jets of water produced during jet-propulsive swimming are effected by exaggerated expiratory movements and knowledge of this dates back at least to Tonner (1936). Aspects of ventilation and swimming have been reviewed

by Mill (1972, 1974, 1977a,b. 1982b, 1985, 1997), Mill & Pickard (1972a), Komnick (1982) and Komatsu (1984b). The increase in internal body pressure that effects the onset of labial mask extension and the details of that extension have been studied by Pritchard (1965, 1976), Olesen (1972, 1979) and Tanaka & Hisada (1980).

The odonate nervous system consists of a brain, a sub-oesophageal ganglion, three thoracic ganglia and eight abdominal ganglia, all joined together by paired connectives

Before dealing with ventilation, jet-propulsion and labial mask ejection, a brief review of the structure and function of nerves and muscles is given to help with interpretation.

Types of nerve cells (neurons)

All nerve cells (neurons) have a cell body bearing small processes called dendrites which serve to receive information from, and transmit information to, other neurons. Some neurons also have a long process called an axon which, as explained below, is necessary for the transmission of information over long distances. It is important to distinguish between neurons and nerves, the latter comprising processes (axons) of the former. There are three types of neurons:

- Sensory neurons. These convey information from sense organs to the central nervous system. As in all insects, the cell body lies in the periphery and the dendrite(s) is/are associated with the sense organs (hairs, spines, stretch receptors and chordotonal organs). Their axons run in nerves to the central nervous system.

- Interneurons. These are contained entirely within the central nervous system. Some collect information from the sensory neurons, others transmit information around the central nervous system, while yet others control the motor neurons. Their form is extremely varied. Some are very small and contained within a single ganglion, others have processes in more than one ganglion and yet others have an axon that runs through most/all of the ganglia, giving off side branches in each ganglion through which it passes.

- Motor neurons. The cell bodies of motor neurons are located within the central nervous system and they receive information from interneurons via their dendrites. Each motor neuron has an axon which runs in a nerve and transmits information to muscle fibres (cells).

How neurons work.
The interior of a neuron has a small negative potential (in the region of at most a few tens of mV) with respect to the outside. The dendrites pass information from cell to cell via small graded potentials. Some of these potentials produce a decrease in the internal negativity of the receiving cell (**depolarising** potentials); others cause an increase in its negativity (**hyperpolarizing** potentials). The total input being received by a cell is collated and, if the cell is one which has an axon, and if the depolarisation reaches a certain threshold level, an action potential is elicited in the axon. What happens when this threshold is reached is that a chain reaction is triggered which causes the inside of the cell to rapidly depolarise further, with the inside briefly becoming positive with respect to the outside. Once produced, the action potential(s) self-propagates along the cell's axon to its destination (an interneuron in the central nervous system if it is a sensory neuron, other interneurons or motor neurons if it is an interneuron, or with muscle fibres (cells) if it is a motor neuron). The size of the action potential is in the region of tens of millivolts and remains constant for a given axon; it is generally larger in larger diameter axons. The reason why action potentials are necessary is that the small graded potentials decay over distance from their origin and hence are unable to convey information over any appreciable distance.

Action potentials arriving in the axon of a motor neuron at a muscle depolarise the muscle fibres that they innervate to produce muscle potentials. These potentials tend to be longer lasting than the action potentials in axons. The greater the frequency of the action potentials in the motor neuron the greater the muscle contraction. However, a single action potential in a motor neuron is unlikely to cause any muscle contraction. If the motor neuron fires (produces action potentials) repetitively and if the action potentials are very close together the muscle potentials add to each other, a phenomenon known as summation. However, even if they are too far apart to cause summation they may cause facilitation, whereby the second action potential causes a larger muscle potential that the first and so on up to a maximum.

Techniques

Movemnts of the floor of the abdomen (sterna) (Hughes & Mill, 1966; Pickard & Mill, 1972) and of the labial mask (Tanaka & Hisada, 1980) have been recorded using a light source and a phototransistor, arranged so that any movements interrupted the light beam and hence the amount of light received by the transducer. The changes in light intensity were converted into electrical signals, amplified and viewed on an oscilloscope.

Cinematography has been used to measure movements of the anal valve during respiration (Pickard & Mill, 1975) and the labial strike (Pritchard, 1965; Olesen, 1979; Tanaka & Hisada, 1980).

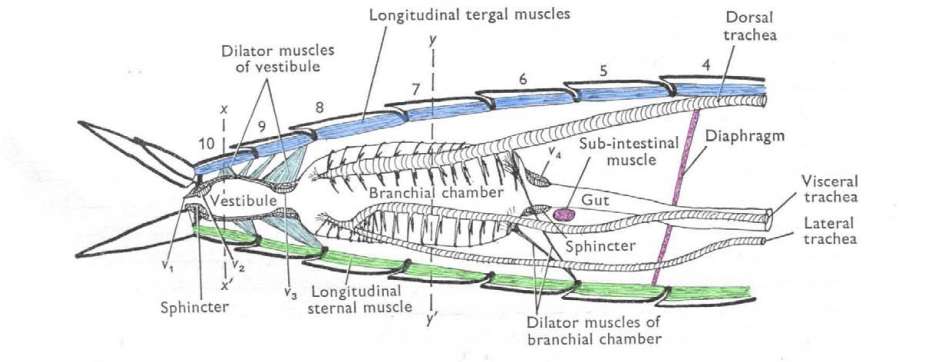
Pressure changes in the branchial chamber have been recorded by inserting a fine, steel hypodermic needle (Hughes & Mill, 1966) or a piece of polyethylene tubing (Tanaka & Hisada, 1980) through the anus and vestibule into the chamber and connecting it to a pressure transducer. Similarly, haemolymph pressure has been recorded by Oleson (1972, 1979) using a steel hypodermic needle inserted dorsally between the wing buds or above a prothoracic or mesothoracic coxa.

An isometric strain gauge attached to the wing buds via an aluminium rod was used to measure forces exerted during jet-propulsive swimming (Mill & Pickard, 1975).

Recordings from nerves in dissected preparations were obtained by using fine platinum wire electrodes placed under the nerves (e.g. Mill & Hughes, 1966) or by using suction electrodes (Olesen, 1979). Since different diameter axons tend to have different sized action potentials (see above) it is often possible to distinguish the activity in individual axons when recording from a nerve containing several. Because the recording electrodes are on the outside of the axon the size of the action potentials recorded were reduced from tens of millivolts to microvolts. The potential changes were amplified and recorded as 'spikes' (vertical deflections) on an oscilloscope. In some cases, where a neuron has a large diameter axon, it has been possible to insert a glass microelectrode containing a saline solution into it (Komatsu, 1980, 1984a, b), in which case the recorded action potentials are their 'proper' size.

As noted in the first part of this review (Mill, 2016), insect muscles are innervated by a very small number of motor neurons, typically between one and five. Recordings were made from the muscles of dissected preparations by placing fine platinum wire electrodes on their surface. Muscle recordings are a bit more difficult to interpret than those from nerves because many muscle fibres will be activated simultaneously by an action potential in a single motor neuron. The recordings obtained from them will thus be the sum of a number of muscle potentials. Further difficulties of interpretation result from summation and facilitation (see above).

Muscle recordings have also been obtained from intact larvae, both restrained and free-moving, by inserting fine, enamel-covered copper wires (Pickard & Mill, 1975; Tanaka & Hisada, 1980) or laquer-covered steel wires (Oleson, 1972, 1979) through the integument close to a muscle attachment point. If the electrodes are removed there is no lasting damage to the larva, which is still



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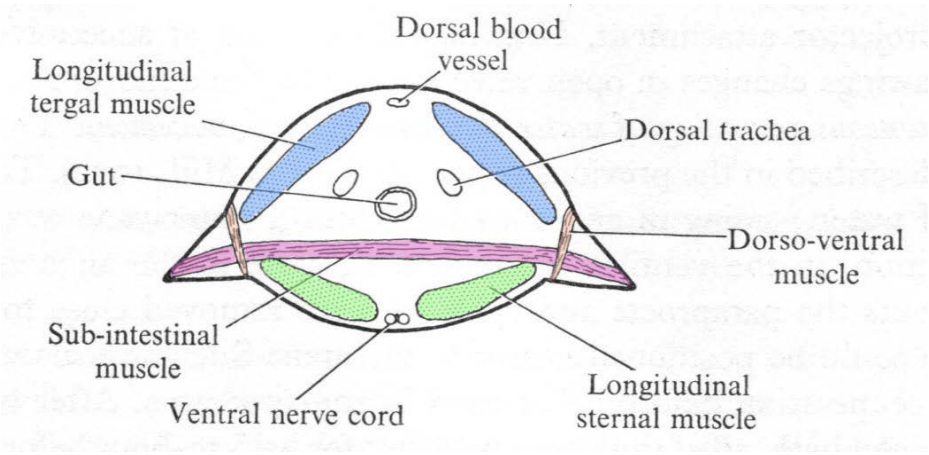


B

Figure 1. Sections through the abdomen of a larval aeshnid. (A) longitudinal section, (B) transverse section in the region of the sub-intestinal muscle; , longitudinal sternal muscles; , longitudinal tergal muscles; , diaphragm and subintestinal muscle; , dorso-ventral muscles; , dilator muscles of the vestibule. From Mill (2016). (A after Hughes & Mill (1966); B after Mill & Pickard (1972)).

able to complete its metamorphosis (Pickard & Mill, 1972).

It can be helpful to stimulate neurons to see what effect this has on various other neurons and muscles. This has been carried out on whole nerves using fine platinum wire electrodes (Mill & Hughes, 1966; Mill, 1970) or suction electrodes (Olesen, 1979). Komatsu (1980) stimulated large diameter interneurons intracellularly using a glass microelectrode.

Ventilation

Mechanics

There has been considerable interest in the role of the abdomen in ventilation for a long time (e.g. Poupart, 1702; Dufour, 1852; Amans, 1881; Matula, 1911). Normal ventilation involves the floor of the abdomen (sterna) being raised and lowered repetitively, water being ejected from the anus during sternal lifting and taken in during sternal lowering (Babak & Foustka, 1907; Wallengren, 1914; Whedon, 1918; Tonner, 1936; Hughes & Mill, 1966). When an aeshnid larva is at rest the branchial chamber is full of water, the floor of the abdomen (sterna) is fully distended and the anal valve is closed.

Expiration The first phase of ventilation is expiration. The sterna are raised by the contraction of the abdominal respiratory (expiratory) dorso-ventral (rdv) muscles (Fig. 1) (Mill, 1965; Mill & Hughes, 1966), the movements being greatest in segments 5 to 7 (Pickard & Mill, 1974). This increases the internal pressure in the abdominal cavity, which causes an increase in the pressure within the branchial chamber (Figs 1, 2). As the sterna start to lift, the anal valve opens to about one third of its maximum (Fig. 3) and the pressure in the branchial chamber thus forces the contained water out of the anus (Mill & Pickard, 1972b). The increase in branchial chamber pressure may possibly be augmented by contraction of its intrinsic muscles. In this phase the pressure inside the branchial chamber normally reaches between 2 and 5 cm H_2O (Fig. 2) (Hughes & Mill, 1966). Olesen (1979) recorded a similar rise in pressure (3 cm H_2O) in the thoracic haemolymph of *Cordulia aenea*. When the sterna are fully lifted the anal valve opens completely and the pressure in the branchial chamber falls to zero (Fig. 2) (Mill & Pickard, 1972b). This marks the end of expiration.

Inspiration Inspiration is effected by the sterna returning to their resting position (Figs 2, 3). This is achieved by the contraction of the diaphragm (first described by Amans (1881) and sub-intestinal muscle (Fig. 1) along with the effect of the natural skeletal elasticity of the abdomen (Tonner, 1936; Hughes & Mill,

1966). There may also be some contraction of the vestibular dilator muscles. The net effect is to produce a negative pressure of 0.5 – 1.0 cm H_2O in the branchial chamber (Fig. 2) (Hughes & Mill, 1966). With the anal valve still fully open, water is drawn in through the anus. The anal valve then closes and the ventilation cycle is complete (Fig. 3) (Mill & Pickard, 1972b).

A complete cycle generally lasts about 1 sec (Hughes & Mill, 1966). There is a brief pause between ventilatory cycles and it is the length of this pause that affects the frequency of ventilation, which generally varies between 23 and 48 cycles/minute in aeshnid larvae but is somewhat higher in *Libellula* sp. (55-90 cycles/minute) (Hughes & Mill, 1966) and in *Cordulia aenea* (mean = 56 cycles/minute) (Olesen, 1979).

The partial closing of the anal valve during expiration and the relatively high pressure in the branchial chamber causes the water to be ejected some distance from the anus, whereas the full opening of the anal valve and the relatively low negative pressure in the branchial chamber during inspiration pulls water in from close to the anus. This reduces mixing of the expired and inspired water (Mill & Pickard, 1972b).

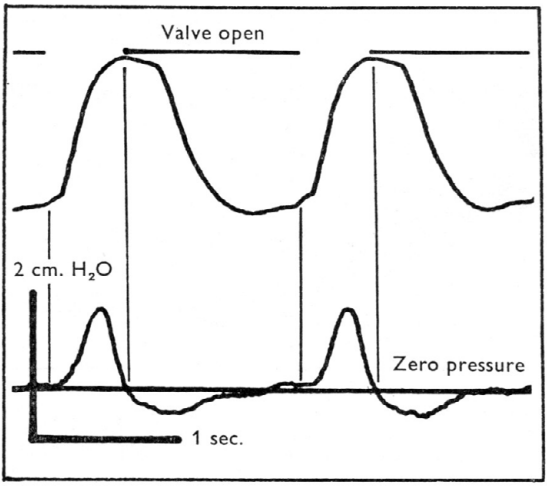


Figure 2. Ventilation. Two cycles of the dorso-ventral movements of the sternum (upper trace) and of pressure changes within the branchial chamber (lower trace). The time when the anal valve is open is indicated by the horizontal bars at the top (see Fig. 3 for more detail).The vertical scale bar refers to the pressure changes. From Hughes & Mill, 1966.

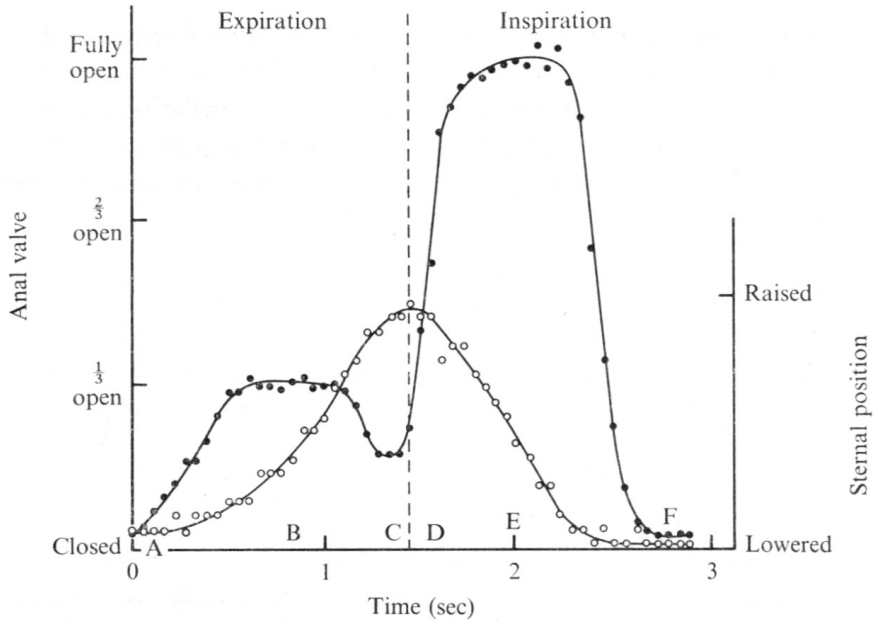


Figure 3. Ventilation. Open valve area (●) and sternal movement (○) in a single ventilatory cycle. Measurements taken from projected ciné-frames. From Mill & Pickard1972.

The above account is of normal, rhythmic ventilation. However, occasionally other ventilatory movements occur, as first described by Tonner (1936). These include 'Gulping Ventilation', when the pressure in the branchial chamber increases to 10cm – 30cm H_2O and is maintained for about 10 seconds (Hughes & Mill, 1966; Pickard & Mill, 1972), and 'Chewing Ventilation', which comprises small pressure changes in the branchial chamber during gulping ventilation (Hughes & Mill, 1966). These will not be considered any further in this review.

Physiology

The second segmental nerves (n_2) innervate the respiratory dorso-ventral (rdv) muscles, which are the muscles which lift the sterna to effect expiration, while the unpaired nerves in abdominal segments 5 and 6 innervate the sub-intestinal muscle and diaphragm respectively to help force the sterna downwards, eliciting inspiration (Fig. 1). The alternation of expiration and inspiration can be observed by recording from the appropriate nerves and/or muscles (Fig. 4). Much of the work has been carried out on dissected preparations (e.g. Mill & Hughes, 1966; Mill, 1970; Komatsu, 1980) but work on intact preparations has confirmed and extended these findings (e.g. Pickard & Mill, 1972, 1975).

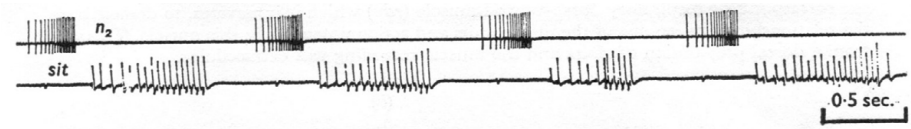
Expiration During expiration in *Aeshna* the expiratory bursts normally contain just one active motor neuron in the second segmental nerves (n_2) (Fig. 4A,B), although occasionally a second one may be active, whereas in *Anax* there are usually three active motor neurons (Mill, 1970). This difference has been confirmed by Komatsu in *Aeshna nigroflava* and *Anax parthenope* (pers. comm.). However, in *Anax* only one of the motor neurons causes potentials in the respiratory dorso-ventral (rdv) muscle (Fig. 5) (Mill, 1970).

Typically, the expiratory motor neuron shows an increase in the frequency of action potentials from about 10-20/second at the start of the burst to a maximum of about 100/second and then the firing rate decreases rapidly at the end of the burst (Fig. 4A,B) (Mill & Hughes, 1966; Mill, 1970). The duration of the expiratory burst lasts from about 0.2 sec to almost 1 sec and the number of action potentials from 7-30 (Mill & Hughes, 1966). The expiratory bursts are in phase on opposite sides of a segment with a pattern that may or may not be 1:1 (Fig. 6) (Mill & Hughes, 1966; Pickard & Mill, 1972). The expiratory burst finishes about 100 msec before the sterna reach their fully raised position (Fig. 7) (Pickard & Mill, 1972).

Each action potential in the expiratory motor neuron elicits a muscle potential in the fibres of the respiratory dorso-ventral (rdv) muscle and, as the frequency of nerve action potentials increases, the muscle potentials increase in amplitude, a process known as facilitation (see above) (Fig. 8) (Mill, 1970). The tension in an individual rdv muscle reaches a peak at the same time that the expiratory burst ends (Fig. 9) (Pickard & Mill, 1972).

The expiratory bursts in the second segmental nerves first start in the eighth abdominal segment and then appear progressively in more anterior ones at least as far as segment 5, indicating that the ventilatory rhythm is initiated in the eighth abdominal ganglion and spreads forwards (anteriorly). The bursts in different segments reach similar maximum frequencies and end at about the same time, about 100 ms before the sterna are fully raised (Fig. 10) It is the inertia generated that causes the sterna to continue moving up (Pickard & Mill, 1972). The intersegmental delay varies between about 50 ms and 150 ms per segment (Mill & Hughes, 1966; Pickard & Mill, 1972).

The extra motor neurons in the second segmental nerve (n_2) probably serve to maintain tension in the other dorso-ventral muscle that it innervates (the anterior dorso-ventral (adv) muscle). Activity has also been recorded during expiration in the first (n_1) and third (n_3) segmental nerves (Fig. 11). which innervate respectively the anterior dorso-ventral (adv) and longitudinal (lt) muscles, and the posterior dorso-ventral (pdv) and posterior tergo-pleural (ptp) muscles (Mill, 1970; Pickard & Mill, 1975). Activity has been recorded from the anterior dorso-



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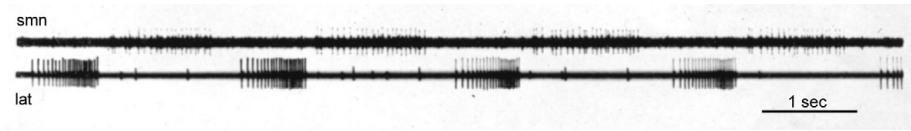

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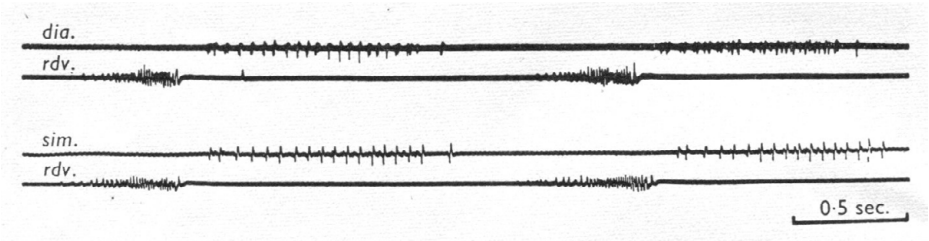


C

Figure 4. Ventilation. The alternation of expiration and inspiration. (A) the upper trace (n_2) is recorded from a second lateral nerve (expiratory) of 7th abdominal ganglion; the lower trace (*sit*) is from the subintestinal muscle (inspiratory), (B) the upper trace (*smn*) is recorded from the branch of the median nerve innervating the sub-intestinal muscle; the lower trace is from a second lateral nerve (*lat*) of 7th abdominal ganglion, (C) the upper traces are recorded from the diaphragm (*dia*) and sub-intestinal (*sim*) muscle (inspiratory) respectively; the lower traces are from a respiratory (expiratory) dorso-ventral muscle (*rdv*) of the 8th abdominal segment. A from Mill (1970); B, C from Mill & Hughes (1966).

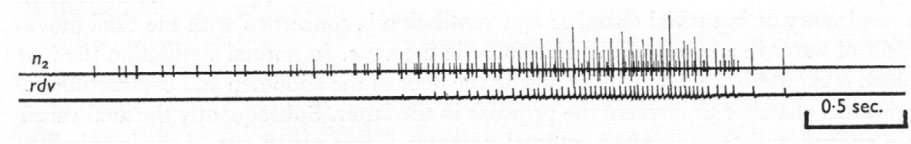


Figure 5. Ventilation. The upper trace (n_2) is recorded from a second lateral nerve of the 7th abdominal ganglion in *Anax imperator*; the lower trace is recorded from the corresponding respiratory (expiratory) dorso-ventral muscle (*rdv*) that it innervates. Note that only one of the motor units in n_2 elicits potentials in the rdv muscle. From Mill (1970).

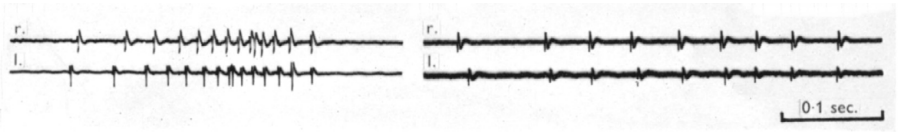


Figure 6. Ventilation. Recordings of expiratory bursts from the second segmental nerve (n_2) from the right (r) and left (l) sides of 6th abdominal ganglion. Note the 1:1 relationship between the potentials in (B) but not in (A). From Mill & Hughes (1966).

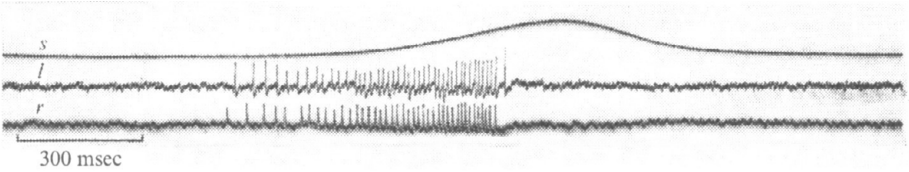


Figure 7. Ventilation. An expiratory burst in the right (*r*) and left (*l*) respiratory dorsoventral muscles of 6th abdominal segment. Upward deflection of the top trace (*s*) indicates lifting of the floor of the abdomen (sterna). From Pickard & Mill (1972).

ventral (adv) and longitudinal (lt) muscles during expiration (Fig. 12); also the ventral adductors of the anal appendages.

The rising abdominal pressure could potentially cause abdominal extension, reducing the effect on the branchial chamber. However, this does not occur since, during expiration, the activation of the longitudinal muscles is presumably sufficient to increase the tension in them, thereby preventing any such extension, but not sufficient to cause any abdominal shortening (Mill, 1970; Pickard & Mill, 1975). There is also expiratory activity in the unpaired nerve sn_{10}, the active neurons probably innervating muscles of the branchial chamber (Mill, 1970). The posterior dorsoventral (pdv), lateral primary longitudinal sterno-pleural and dorso-ventral oblique (dvo) muscles show no activity during ventilation. In free-swimming larvae muscle potentials in the rdv muscles during expiration tend to be somewhat more variable than in dissected and restrained preparations (Pickard & Mill, 1975), possibly due to the exposure of free-swimming larvae to sensory inputs.

Inspiration. The inspiratory motor neurons in the unpaired nerves (sn) innervating the diaphragm and sub-intestinal muscle also fire rhythmically in bursts but, within a burst, they fire at a fairly steady frequency (Mill & Hughes, 1966; Mill. 1970) (Fig. 4). It has been shown that these motor neurons receive inhibition during expiration (Komatsu & Kusachi, 1979; Komatsu, 1980).

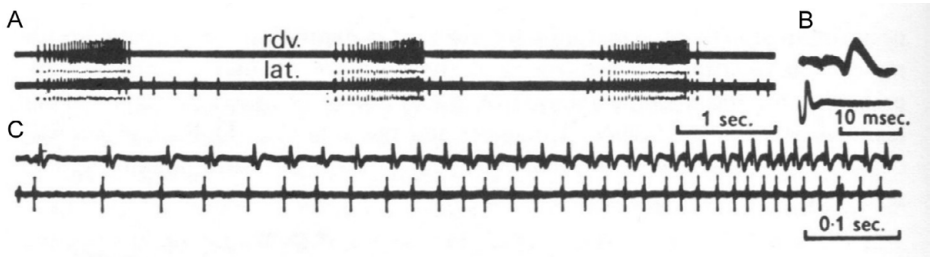


Figure 8. Ventilation. (A) three expiratory bursts recorded from one side of 7th abdominal segment. The lower trace is a recording from a second lateral nerve (lat); the upper trace shows the corresponding extracellularly recorded muscle potentials from the respiratory dorso-ventral muscle (rdv) that it innervates. Note the effect of facilitation, whereby the muscle potentials increase in size to a maximum during the burst; also the presence of action potentials from at least one other neuron that are not associated with any muscle potentials, (B) same preparation showing superimposed potentials from a single burst to show the consistent delay between the nerve and muscle potentials. (C) same preparation showing a single burst on an expanded time-scale. From Mill & Hughes (1966).

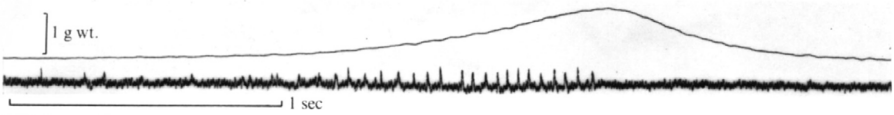


Figure 9. Ventilation. Activity recorded from the right respiratory dorsoventral (rdv) muscle of the 6th abdominal segment (lower trace) and the strain on the pleuron to which the muscle is attached (upper trace). From Pickard & Mill (1972).

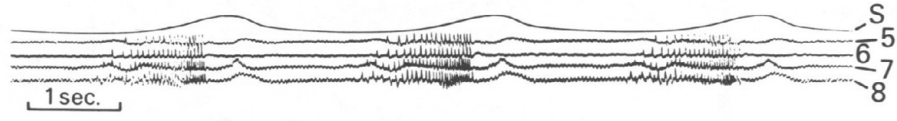
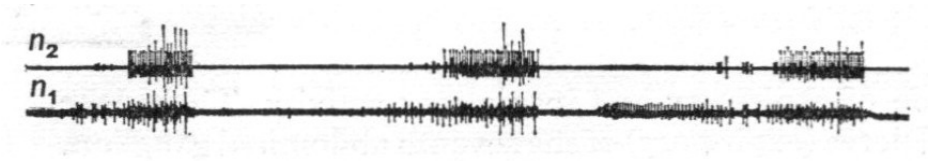


Figure 10. Ventilation. Expiratory bursts recorded from the right dorsoventral (rdv) muscles of 5th-8th abdominal segments (5-8) showing the serial staggering of the bursts from behind forwards. Upward deflection of the top trace (s) indicates lifting of the floor of the abdomen. From Pickard & Mill (1972).



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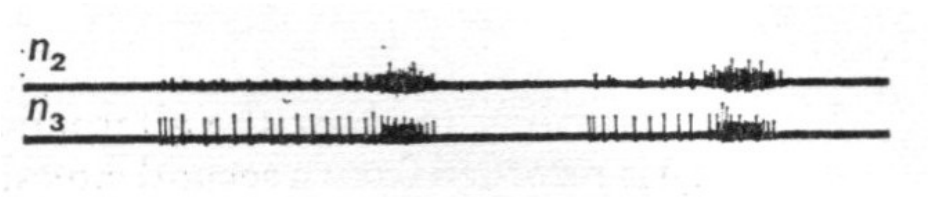

B

Figure 11. Ventilation. Expiratory bursts recorded (A) from a second (n_2) and first (n_1) lateral nerve of 7th abdominal ganglion, (B) from a second (n_2) and third (n_3) lateral nerve of 7th abdominal ganglion. From Mill (1970).

At least in the nerve to the sub-intestinal muscle two motor neurons appear to be active and the action potentials on the two sides of the segment coincide (Mill & Hughes, 1966) (Fig. 13). This is commensurate with the finding of Zawarzin (1924a) that there are two motor neurons entering the unpaired nerve and that both of these divide, sending a branch to each side of the segment. The bursts in the nerves innervating the diaphragm and the sub-intestinal muscle are synchronised (Mill & Hughes, 1966) (Fig. 4C).

There is a very close coupling between the expiratory and inspiratory bursts with a delay between them of 100-150 ms. The pause between inspiration and expiration is longer and variable and hence determines the ventilatory rate (Mill & Hughes, 1966). The above information is summarised in figure 14.

Neural Control. The first segmental nerve (n_1) contains the sensory neurons of the two stretch receptors and the chordotonal organ, as well as those of the external receptors on the tergum. When it is electrically stimulated in the interval between expiratory bursts, a burst similar to that of a normal expiratory burst is elicited in the second segmental nerve (n_2) of the same segment and in n_2 of more anterior segments (Fig. 15) (n_2 is the segmental nerve that contains the axon that innervates the corresponding rdv muscle to effect expiration).

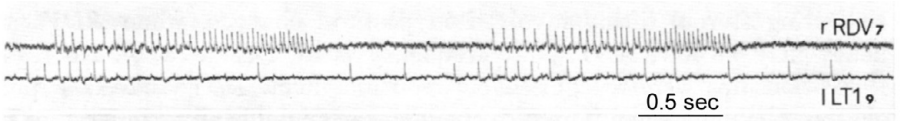

Figure 12. Ventilation. Expiratory bursts recorded from the right dorsoventral muscle of 7th abdominal segment (r RDV_7) and the primary longitudinal tergal muscle of 9th abdominal segment (l $LT1_9$). From Pickard & Mill (1975).

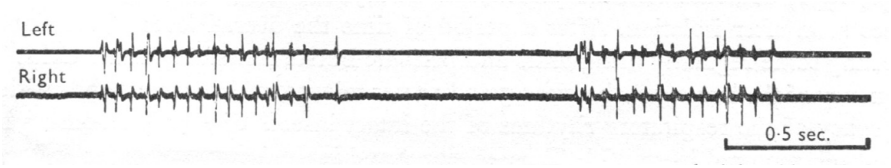

Figure 13. Ventilation. Muscle action potentials recorded from the left and right sides of the sub-intestinal transverse muscle (inspiratory) during two consecutive inspiratory bursts. Note the similarity of the two sides due to the motor neurons in the median nerve dividing to stimulate both sides. From Mill & Hughes (1966).

Thus the ventilatory rhythm is reset (Mill & Hughes, 1966; Komatsu & Kusachi, 1979). Repetitive stimulation at a frequency higher than that of the ventilatory rhythm may increase the frequency of the rhythm (Mill, 1970) (Fig. 16). This implies that the centrally generated rhythm may possibly be affected by input from sensory receptors. On cessation of stimulation the rhythm returns to its original frequency (Mill, 1970). Stimulation of n_1 during an expiratory burst has no effect on the ventilatory rhythm (Mill & Hughes, 1966).

Some large diameter (12-16um) interneurons pass through at least most of the abdominal ganglia, giving off branches in each ganglion (Zawarzin 1924b; Hughes, 1953). Amongst these are the Ascending Expiratory (AE) interneurons that have their cell bodies in the last (8th) abdominal ganglion, the axon of each one running forwards on the same side as its cell body and giving off branches in at least 7th – 4th abdominal ganglia (Komatsu & Kusachi, 1982; Komatsu, 1984a). Intracellular recording from the axon of an AE interneuron shows bursts of action potentials that are in phase with expiration, the burst in an AE interneuron starting about 150 msec before the corresponding expiratory burst in a second segmental nerve (n_2) (Fig. 17) (Komatsu, 1984a). Not only can stimulation of a segmental nerve reset the ventilatory rhythm in the expiratory motor neurons if the stimulus is applied in the interval between successive expiratory bursts (see above), it can also reset the respiratory rhythm in the Ascending Expiratory (AE) interneurons (Fig. 18) (Komatsu & Kusachi, 1982; Komatsu, 1984a).

Although intracellular stimulation of an AE interneuron (using a depolarising current) may affect the activity of motor neurons in a second segmental nerve (n_2), for example, by slightly increasing the length of the expiratory bursts, it does not affect the ventilatory rhythm either of the AE interneuron itself or of the expiratory motor neurons (Fig. 19). Hence these AE interneurons are not involved in driving the rhythm (Komatsu, 1984a). Also, such stimulation has no effect on the inspiratory bursts (Komatsu, 1984a).

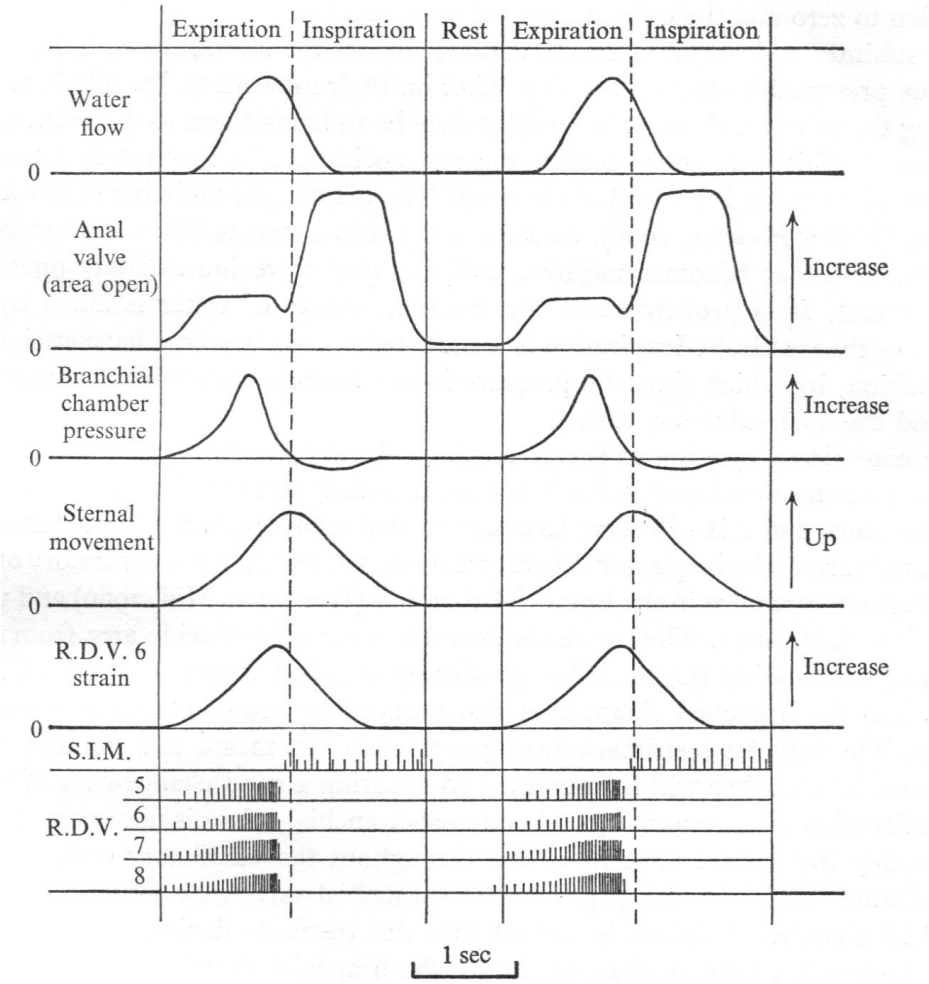

Figure 14. Ventilation. Summary chart of the events occurring in normal ventilation. R.D.V., respiratory (expiratory) dorso-ventral muscle; S.I.M., sub-intestinal muscle (inspiratory); numbers (5-8) refer to the abdominal segments. From Mill & Pickard (1972).

Komatsu (1984b) has also described Ascending Inspiratory (AI) interneuron, the axons of which also pass through several abdominal ganglia but, so far, their cell bodies have not been located. These interneurons show rhythmic bursts of activity that start just before the inspiratory bursts in the median nerves. Stimulation of a segmental nerve during an inspiratory burst suppresses the activity in the interneuron and resets the respiratory rhythm (Komatsu, 1984b).

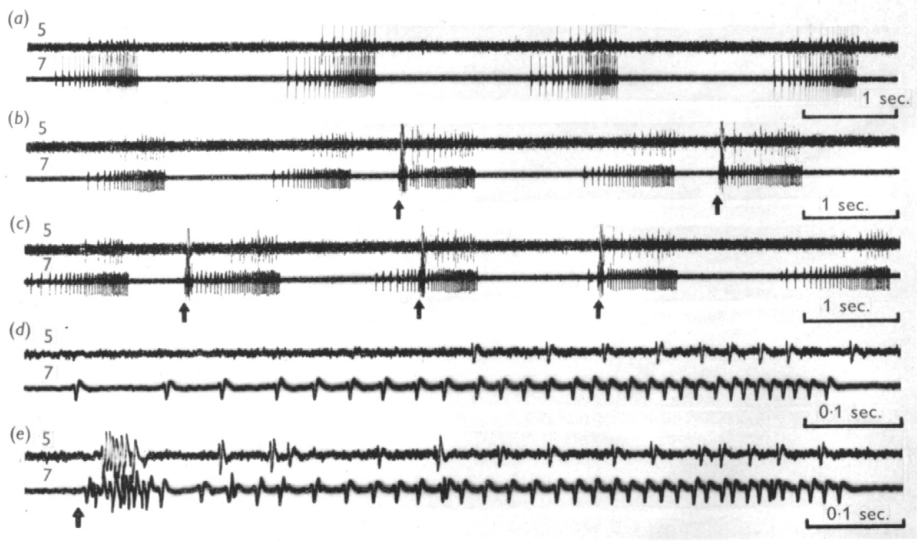

Figure 15. Ventilation. Expiratory bursts recorded from the second segmental nerves on one side of 5th (5) and 7th (7) abdominal segments. (a) Normal ventilation, (b, c) The effect of electrical stimulation of the first segmental nerve of 7th abdominal segment on the same side. Note that an expiratory burst is only elicited when the stimulus is applied between expiratory bursts, (d) a normal burst, (e) an elicited burst. From Mill & Hughes (1966).

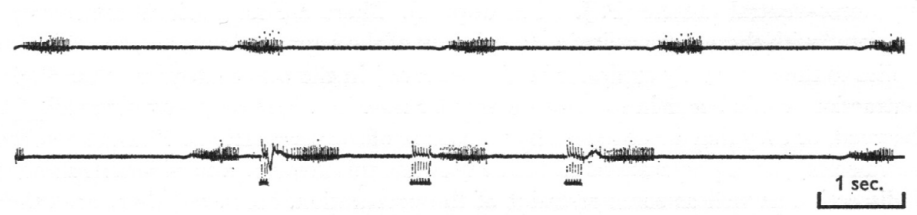

Figure 16. Ventilation. Expiratory bursts recorded from a second segmental nerve of 6th abdominal ganglion. Note the increase in expiratory burst frequency when the first segmental nerve on the opposite side is stimulated repetitively at a frequency higher than the normal burst frequency. The traces are continuous. From Mill (1970).

Jet-propulsive Swimming

Mechanics

Jet-propulsive swimming in aeshnid larvae is achieved by the very rapid expulsion of a jet of water from the anus (i.e. an exaggerated expiratory movement) coupled with the legs being drawn in alongside the body and pointing backwards to improve the streamlining of the larva. Jet-propulsion may starts either abruptly or following one or more vigorous ventilatory movements. Following a period of jet-propulsive swimming there are normally a few cycles of strong ventilation (Mill & Pickard, 1975). In some cases there is just a single cycle but the frequency can reach up to 2.2 cycles/sec. Unlike in ventilation the abdomen is shortened at the same time that the floor of the abdomen is raised. This combination of sternal lifting and abdominal shortening results in a much higher pressure within the branchial chamber (about 30-40 cm H_2O) than is achieved during ventilation and the rate of increase in pressure is also higher (Hughes & Mill, 1966). The internal pressure recorded in the thorax appears to be even higher, reaching up to 100 cm H_2O (Fig. 20) (Olesen, 1972, 1979). Water is thus ejected more rapidly through the (partially open) anal valve, propelling the larva through the water. This is aided by adduction of the paraprocts and epiproct which channel the expired water into a narrow jet (Mill & Pickard, 1975). The maximum swimming velocity is about 10 cm/sec, while the maximum thrust recorded for individual cycles is between $9.8x10^{-3}$ N and $14.7x10^{-3}$ N (Mill & Pickard, 1975).

Physiology

During jet propulsive swimming, the respiratory dorso-ventral muscles (rdv) show a short burst of activity (100-400 msec in length). More axons are active than in expiration and the frequency of action potentials in the bursts is higher (Figs 20, 21) (Olesen, 1972; Mill & Pickard, 1975). Generally there is an increase in the frequency within the expiratory bursts prior to jet propulsion occurring and again for a few bursts after swimming has ceased (Fig. 21). There is not such a precise symmetry between left and right sides, as is found in expiration, but the bursts still begin and end together. No delay between the start of bursts in successive segments has been recorded (Mill & Pickard, 1975). The anterior dorso-ventral (adv) muscles are also very active during jet-propulsion (Olesen, 1972; Mill & Pickard, 1975) and Olesen (1972) noted that there were, on average, 10 muscle potentials during a burst of 120 msec duration (Fig. 20). The activity in the adv muscles coincides closely with that in the rdv muscles (Fig. 22). There was also some activity in the posterior dorso-ventral (pdv) muscles.

Shortening of the abdomen is effected by contraction of both longitudinal and oblique muscles, which occurs in synchrony with the activity in the dorso-ventral muscles. This activity has been confirmed for the primary and secondary longitudinal tergal (lt_1 and lt_2), lateral primary longitudinal sterno-pleural ($llsp_1$)

and dorso-ventral oblique (dvo) muscles (Fig. 23). The adductor muscles of the paraprocts also contract during jet-propulsion (Mill & Pickard, 1975).

Following each burst of activity in the dorso-ventral and longitudinal muscles, there is a burst of activity in the sub-intestinal muscle. The potentials in this burst are of much higher frequency than during inspiration; this activity helps to restore the resting position before the next jet-propulsive burst occurs (Mill & Pickard, 1975).

Prey Capture

Mechanics

Prey capture is achieved by the rapid anterior projection (protraction) of the labial mask and involves extension of both the head-postmentum and the postmentum-prementum joints, together with opening and closing of the labial palps (Fig. 24). For last instar *Aeshna emerita* the average strike distance is 4.8 mm (2.5-9.0 m).

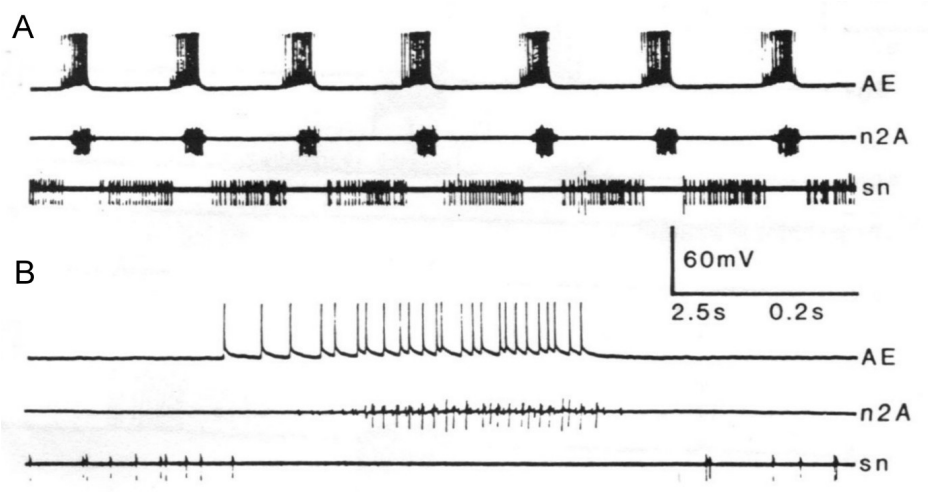

Figure 17. Ventilation. Activity recorded Intracellularly from an ascending expiratory (AE) interneuron in the 7th abdominal ganglion; also expiratory bursts recorded from a second segmental nerve of 5th abdominal ganglion (n2A) and inspiratory bursts in the lateral branch of the median nerve of 6th abdominal ganglion (sn). (A) a series of seven bursts, (B) a single burst seen with an extended time-base. The vertical scale bar refers to the intracellular potentials in the AE interneuron; the horizontal scale bar: (A) 2.5 sec, (B) 0.2 sec. From Komatsu (1984). Reprinted with permission from Springer-Verlag.

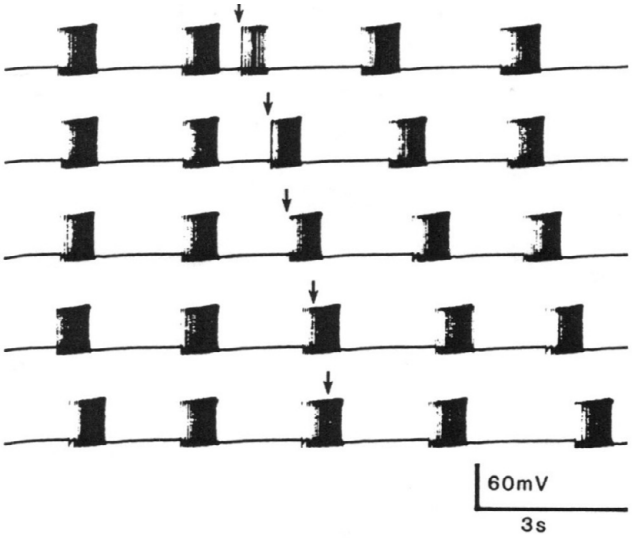

Figure 18. Ventilation. Activity recorded intracellularly from an ascending expiratory (AE) interneuron showing the effect of stimulating a fifth segmental nerve of the 8th abdominal ganglion. The ventilatory rhythm is reset when the stimulus is applied in the period between expiratory bursts. From Komatsu (1984a). Reprinted with permission from Springer-Verlag.

Pritchard (1965) recognised four stages of the labial strike*:* opening of the labial palps, labial extension, closing of the labial palps and labial retraction. The labial palps open before the start of the strike. The time this takes is very variable but on average is between 60 msec (Tanaka & Hisada, 1980) and 80 msec (Pritchard, 1965) in *Aeshna*. Full extension of the labium and closing of the palps (the strike) takes a further 16-40 msec (Pritchard, 1965; Tanaka & Hisada, 1980)). In *Cordulia shurtleffi* the strike is faster (about 15 msec to open the palps and a further 15 msec to extend the labium) and is even faster in *Leucorrhinia hudsonica*, both actions combined taking less than 15 msec (Pritchard, 1965). The speed of labial extension is not temperature dependent (Tanaka & Hisada, 1980). On the other hand, labial retraction is much slower than labial extension (Pritchard, 1965; Tanaka & Hisada, 1980) and is temperature dependent, taking on average in aeshnids 430 msec at 5°C and 100 msec at 20°C (Tanaka & Hisada, 1980).

The anal valve is closed as the labial palps open, while the paraprocts start to close together, complete closure occurring at the start of the strike or within 15-20 ms of its commencement (Pritchard, 1965).

At rest, i.e. when the labial mask is fully flexed, the knobs on the prementum are engaged with grooves on the postmentum (Fig. 24) and so the mask is locked

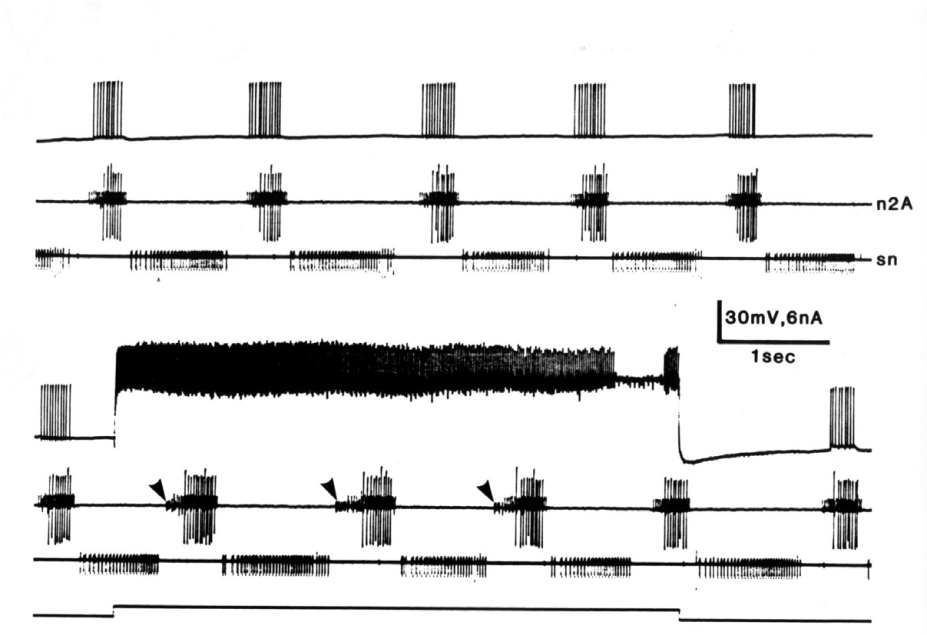

Figure 19. Ventilation. Activity recorded intracellularly from an ascending expiratory (upper trace) interneuron; also expiratory bursts recorded from a second segmental nerve (n2A) and inspiratory bursts in a median nerve (sn). The upper recording (A) is continued in the lower recording (B). (A) a normal sequence, (B) the effect of an application of a long-lasting depolarising current (lowest trace, current monitor), which slightly lengthened the expiratory bursts and caused a small neuron to fire (arrowheads), but which had no effect on the inspiratory bursts or on the ventilatory rhythm. The traces are continuous. From Komatsu (1984). Reprinted with permission from Springer-Verlag.

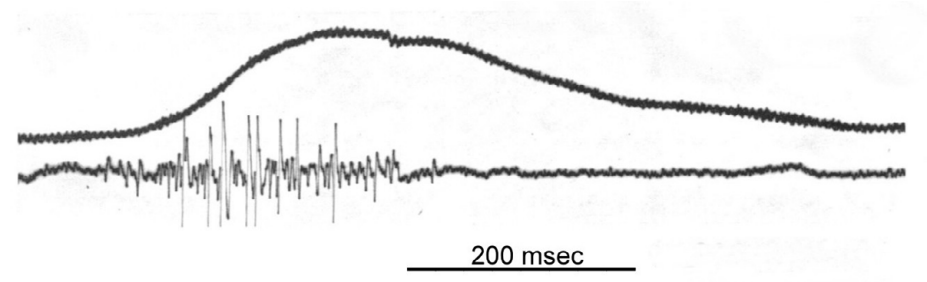

Figure 20. Swimming. Increase in the internal thoracic pressure during jet-propulsive swimming (upper trace) and muscle activity recorded from an anterior dorso-ventral muscle in 4th abdominal segment (lower trace) From Olesen (1972). Reprinted with permission from Springer-Verlag.

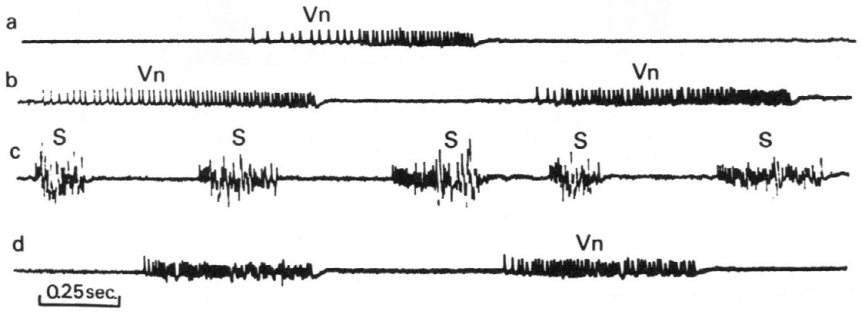

Figure 21. Swimming. Muscle activity recorded from a respiratory (expiratory) dorso-ventral muscle of 7th abdominal segment during normal ventilation (Vn) and jet-propulsive swimming (S) in *Anax imperator*. a-d are continuous. From Mill & Pickard (1972a).

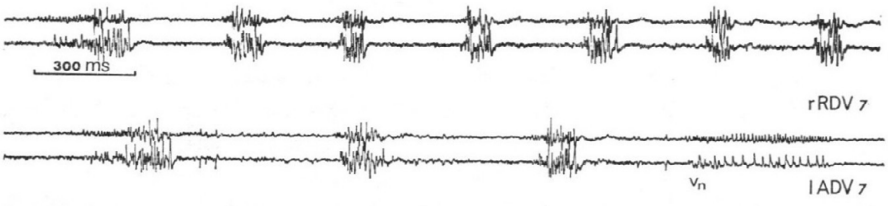

Figure 22. Swimming. Muscle activity recorded from a respiratory (expiratory) dorso-ventral muscle ($rRDV_7$) and an anterior dorsoventral muscle ($lADV_7$) on opposite sides of 7th abdominal segment in an unrestrained larva during jet-propulsive swimming. Note the marked reduction in frequency in the anterior dorsoventral muscle in the first ventilatory movement after swimming. From Mill & Pickard (1975).

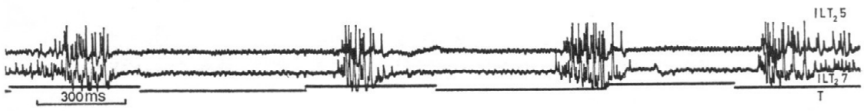

Figure 23. Swimming. Muscle activity recorded from the secondary longitudinal tergal muscles of the same side in 5th (lLT_25) and 7th (lLT_27) abdominal segments during swimming in an unrestrained larva. The bottom trace is a time marker. From Mill & Pickard (1975).

in place (Tanaka & Hisada, 1980). Tanaka & Hisada (1980) described three phases of labial extension after the labial palps are opened. During the first 10 msec the angular velocity of the prementum-postmentum and prementum-head joints increases slowly (angular acceleration 2.6 x 10^5 deg.s^{-2}) to 30° and 50° degrees respectively, the 30° angle of the former being sufficient for the labial knobs to have slid along the grooves and disengage from them. Following their release there is a second phase lasting a further 10 msec during which there is a rapid increase in angular velocity (angular acceleration 7.8 x 10^5 deg.s^{-2}), at the maximum of which the tip of the prementum is moving with a linear velocity of 1.0 m.s^{-1} and the prey is struck. The final phase lasts a further 5 msec during which the labial palps are closed.

The muscles associated with the labial mask are neither large enough nor powerful enough to elicit such rapid movement (Tanaka & Hisada, 1980) and an increase in pressure in the abdomen, brought about by contraction of at least some abdominal muscles, is required to initiate the strike, i.e. it is a hydraulic mechanism (Amans, 1881; Munscheid, 1933; Snodgrass, 1954; Olesen, 1979). The pressure in the branchial chamber starts to increase at anything from 50-350 msec before the start of a strike, reaching a peak of 40-120 cm H_2O at the beginning of the strike (Tanaka & Hisada, 1980) (Figs 25, 26). The increase in the internal pressure in the thorax is similar, Olesen (1979) recording a mean peak pressure of 50 cm H_2O. It is this increase in pressure, coupled with release of the energy stored in the flexor muscles of the prementum (see below), that causes the initial phase of labial extension noted above, resulting in the knobs becoming disengaged from the groves. The rapid extension in the next phase is produced solely by the high internal pressure (Tanaka & Hisada, 1980).

Physiology

Immediately before the start of a strike the following occur:

Abdominal muscles. Starting between 500-110 ms before the start the abdominal respiratory dorso-ventral (rdv) muscles are active, causing lifting of the sterna and thus producing the rise in branchial chamber pressure noted above (Figs 25, 26). Activity ceases 20 ms before the onset of the strike and before maximum pressure is reached (Tanaka & Hisada, 1980). The anterior and posterior (adv and pdv) dorso-ventral muscles are also active during prey capture but the duration of firing, in the former at least, is considerably shorter than in the rdv muscles. Indeed the average muscular activity in the adv muscles comprises only about four potentials in a burst lasting 20 msec (Fig. 27) (Olesen, 1972). In addition, abdominal dorsal and ventral longitudinal muscles are active (Olesen, 1979) but there is not thought to be any abdominal shortening.

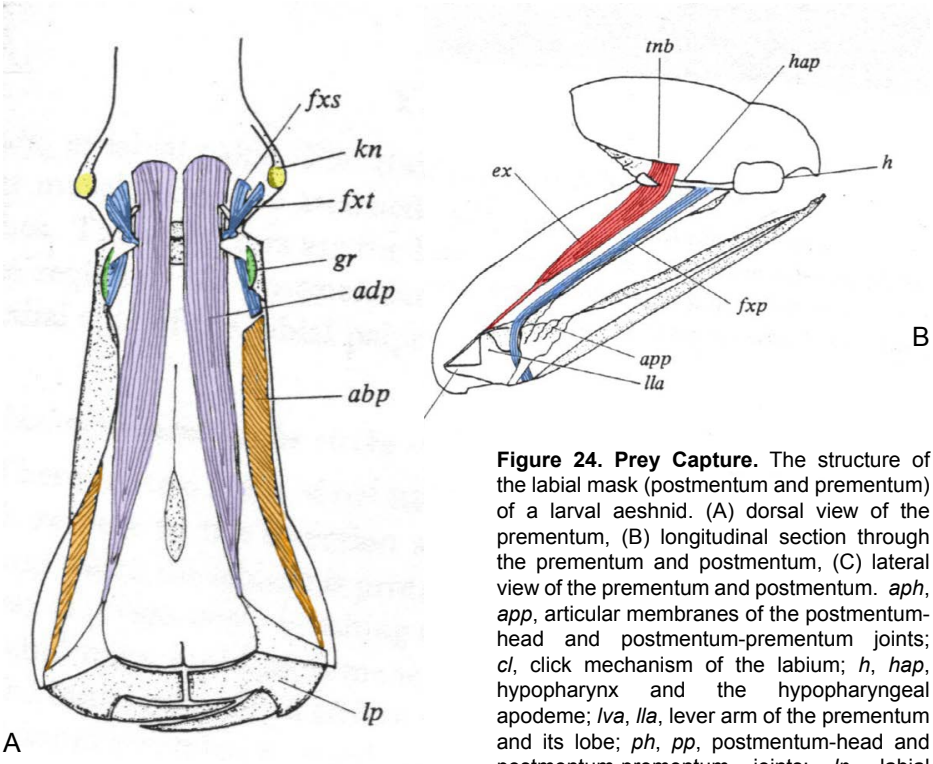

Figure 24. Prey Capture. The structure of the labial mask (postmentum and prementum) of a larval aeshnid. (A) dorsal view of the prementum, (B) longitudinal section through the prementum and postmentum, (C) lateral view of the prementum and postmentum. *aph*, *app*, articular membranes of the postmentum-head and postmentum-prementum joints; *cl*, click mechanism of the labium; *h*, *hap*, hypopharynx and the hypopharyngeal apodeme; *lva*, *lla*, lever arm of the prementum and its lobe; *ph*, *pp*, postmentum-head and postmentum-prementum joints; *lp*, labial palp; *pom*, postmentum; *prm*, prementum; *tnb*, tentorial bar; , flexor muscles of the prementum (*fxp*, *fxs*, *fxt*); , extensor muscle of the prementum; , adductor muscle of the labial palp; , abductor muscle of the labial palp; , *gr*, grooves of the click mechanism; , *kn*, knobs of the click mechanism. After Tanaka & Hisada (1980). With permission from The Company of Biologists.

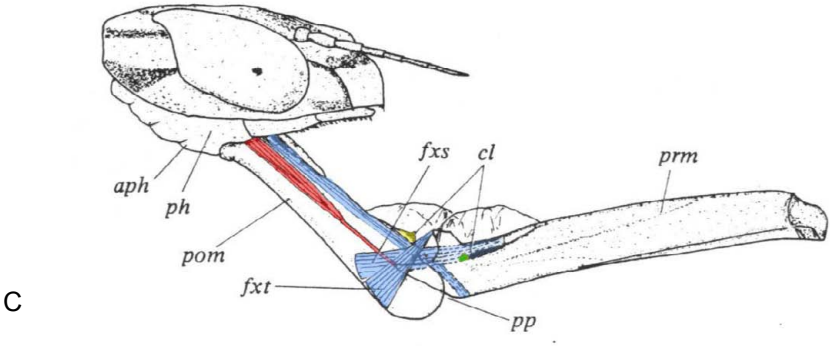

Figure 25. Prey Capture. Muscle activity recorded from an abdominal respiratory dorsoventral muscle (top trace) and branchial chamber pressure (bottom trace) during a labial strike (upwards on the middle trace). Arrow indicates the onset of the strike. Vertical scale bar 100cm H_20; horizontal scale bar 500 msec. From Tanaka & Hisada (1980). Reprinted with permission from The Company of Biologists.

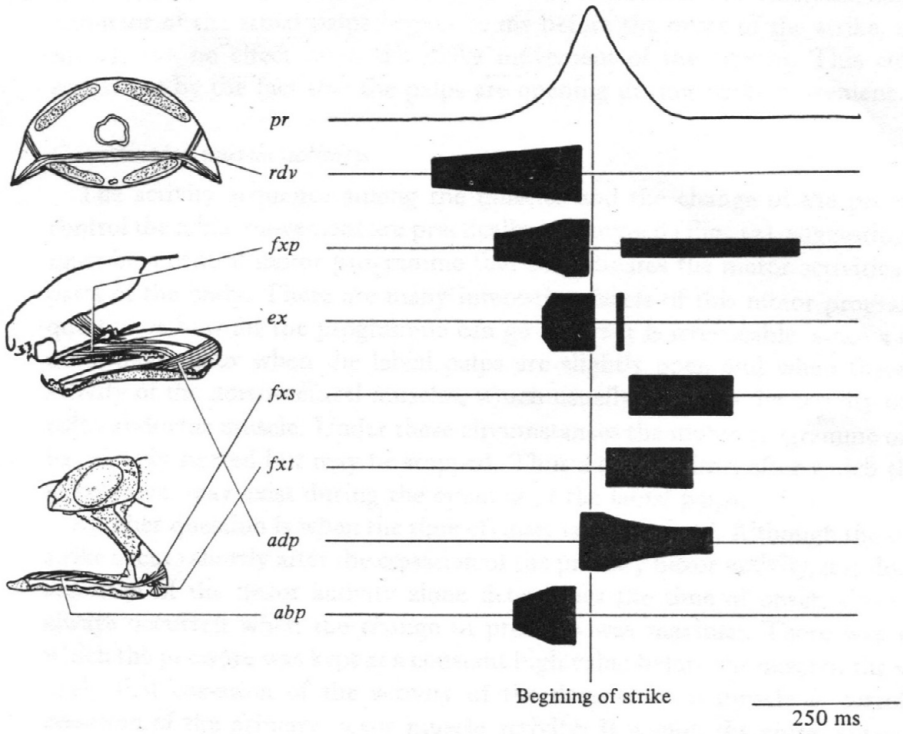

Figure 26. Prey Capture. Summary of branchial chamber pressure (*pr*) and the activity in various muscles involved during a labial strike. *rdv*, respiratory dorso-ventral muscle; *fxp*, primary flexor muscle; *ex*, extensor muscle; *fxs*, secondary flexor muscle; *fxt*, tertiary flexor muscle; *adp*, adductor muscle of the labial palp; *abp*, abductor muscle of the labial palp. From Tanaka & Hisada (1980). Reprinted with permission from The Company of Biologists.

Extensor and flexor muscles of the prementum. 150-50 msec before the start of the strike there is activity in the primary flexor muscles the muscle potentials increasing in frequency and amplitude (Figs 26, 28). The extensor muscles tend to show activity slightly later (about 100-75 msec before the start) (Figs 26, 28). The frequency of potentials in the extensor muscles increases more rapidly and reaches a peak at the same time as that in the flexor muscles; thus they co-contract for about 100 msec before the start of the strike. However, the premental knobs are locked in the grooves of the postmentum, preventing any extension. In this fully flexed (resting) position of the labial mask the primary flexor muscles have a large mechanical advantage over the extensor muscles and hence their co-contraction allows the extensor muscles to contract almost isometrically (i.e. with virtually no change in their length). Activity in the primary flexor muscles normally ends about 5 ms before the start of the strike (Tanaka & Hisada, 1980). In contrast, activity in the extensor muscles continues for 5-20 ms after the start, producing the first phase of labial extension (see above). After full extension is complete, all three flexor muscles become active (Fig. 26), retracting the labium to its rest position.

Abductor and adductor muscles of the labial palps. 125 msec before the start, activity begins in the abductor muscles, the frequency of muscle potentials gradually increasing, opening the labial palps. The burst lasts for about 100 msec and ends before the strike starts. Activity in the adductor muscle starts just 20 msec before the start but continues for about 200 msec.

In summary, the strike is initiated by the end of activity in the primary flexor muscles, which relax. The energy stored in the extensor muscles is thus released and, along with the high internal pressure, produces the first, slow phase of extension, whereby the knobs on the prementum slide along the grooves on the postmentum and disengage from them. The second, rapid phase of extension results solely from the high internal pressure in the body cavity, which produces a large torque to extend the labium with great acceleration. This internal pressure decreases to zero in about 100 ms. (Tanaka & Hisada, 1980).

Summary

An increase in internal body pressure occurs in all three activities. During expiration the peak pressure in the branchial chamber is 2–5 cm H_2O in contrast to the 30-40 cm H_2O recorded in jet-propulsive swimming (Hughes & Mill, 1966) and the 40-120 cm H_2O recorded in prey capture (Tanaka & Hisada, 1980). The pressure recorded in the haemolymph of the thorax was similar for ventilation and prey capture but was higher for swimming (up to 100 cm H_2O) (Olsen, 1979)

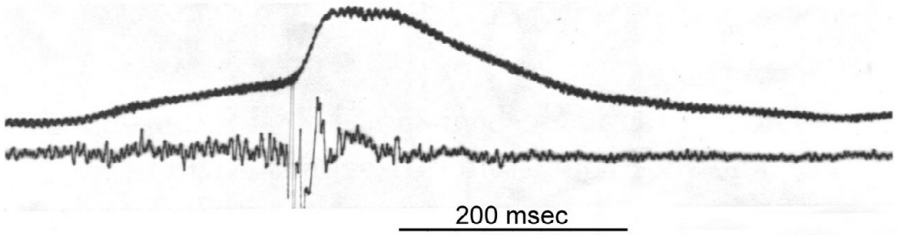

Figure 27. Prey Capture. Increase in the internal thoracic pressure during prey capture (upper trace) and muscle activity recorded from an anterior dorso-ventral muscle in 4th abdominal segment (lower trace). From Olesen (1972). Reprinted with permission from Springer-Verlag.

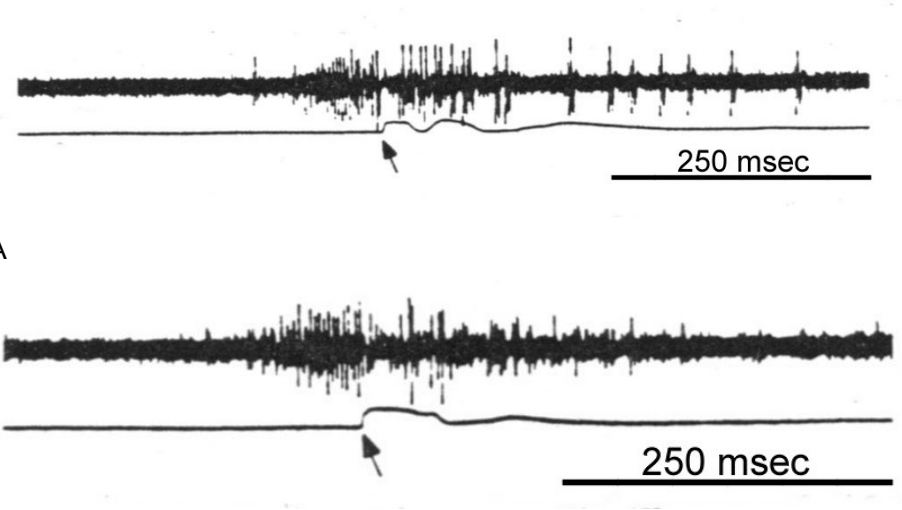

Figure 28. Prey Capture. Muscle activity recorded from (A) a primary flexor muscle of the prementum, (B) an extensor muscle of the prementum (upper traces) during labial strikes (upwards on the lower traces). Arrows indicates the onset of the strikes. From Tanaka & Hisada (1980). Reprinted with permission from The Company of Biologists.

The anal valve opens to about one third of its full extent during expiration, opening fully during inspiration. Similarly the anal valve is only partially open during swimming, which is rather like a rapid expiration. The powerful jet of water produced during swimming is enhanced by the closing of the paraprocts with the epiproct, thereby channelling the water into a narrow jet (Mill & Pickard, 1972b). In prey capture the anal valve is closed and the paraprocts brought together (Pritchard, 1965). This ensures that the effect of the increase in internal abdominal pressure is directed forwards.

During expiration the only dorso-ventral muscle that is active is the respiratory dorso-ventral (rdv) muscle but all three dorso-ventral muscles are active in both swimming and prey capture (Mill & Hughes, 1966; Mill, 1970; Mill & Pickard, 1975. Longitudinal muscles are active in all three activities but shortening of the abdomen only occurs during swimming (Mill & Pickard, 1975; Olesen, 1979). In expiration and prey capture there is an increase in tension in some of the longitudinal muscles to prevent any abdominal extension resulting from the increased internal abdominal pressure since, if this were allowed to occur, it would dissipate the pressure.

In expiration the activity in the respiratory dorso-ventral muscles lasts for 200-1,000 msec (Mill & Hughes, 1966). In swimming the range is 100-400msec (Olesen, 1972; Mill & Pickard, 1975) while in prey capture it is in the region of 400 msec ((Tanaka & Hoisada, 1980).

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