MODELING EXPERIMENTS ON PACEMAKER INTERACTIONS IN SCYPHOMEDUSAE

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TABLE OF CONTENTS

ABSTRACT

Scyphozoan jellyfish are free-swimming gelatinous organisms whose nervous system includes a motor nerve net that controls swimming. Swim contractions originate in a network of distributed pacemakers found in the marginal rhopalia, which are located around the margin of the bell. Many scyphozoan jellyfish have eight rhopalia, while others have sixteen or more. At any one time, the fastest pacemaker controls the output of the swim system. The activity of a single pacemaker is irregular; however, by linking multiple irregular pacemakers the swim system exhibits regular contractions. Thus, multiple pacemakers are believed to increase the frequency and regularity of swim contractions. Pacemaker interactions in *Chrysaora quinquecirrha*, *Stomolophus meleagris*, *Aurelia aurita*, and the ephyra of *Aurelia aurita* were investigated using artificial pacemaker networks created from pacemaker ablation experiments. In all species, with increasing pacemaker number, the frequency and regularity of swimming increased. Integrate and fire pacemaker models were used to determine if the pacemaker networks of three species of scyphomedusae were resetting, independent, or semi-independent. It is concluded that *Chrysaora quinquecirrha* and *Stomolophus meleagris* have a resetting pacemaker networks. In contrast, *Aurelia aurita* has a semi-independent pacemaker network and the ephyra of *Aurelia aurita* has resetting pacemaker network.

ACKNOWLEDGEMENTS

I would thank Mr. Dennis Dudley who got me interested in science while I was in high school. I am especially grateful to Dr. Steven Emslie who introduced me to marine biology, gave me the chance to travel with him to Antarctica, and provided me with endless support and encouragement throughout my college career. Many thanks go to my adviser Dr. Richard Satterlie for putting up with me for two years, for giving me the opportunity to study under him, and for the many biology related and life lessons that he has taught me within these two years.

Special thanks go to my parents and grandparents who helped me along way. In addition, special thanks go to Ms. Samantha Johnson and Dr. Thomas Nolen, for without them this project would have taken more than two years to complete.

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DEDICATION

I would like to dedicate my thesis to five very important people: Henry Brown Sr., Oliva Sirlestine Brown, Frances Hayward, Rodney Hayward, and Sarah Brown Hayward. Their continued support and encouragement have meant more to me than they could ever know. Even though my grandparents (Henry and Oliva Brown) died before I was born, I knew that they were with me every step of the way.

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

BIOGRAPHICAL SKETCH

Rodney T. Hayward was born on April 25, 1983, in Elizabeth City, North Carolina. He graduated from the University of North Carolina Wilmington in May 2005 with a B.A. in Psychology and a B.S. in Marine Biology. He is has worked as research assistant since August 2001 and has traveled to Antarctica were he spent three months studying the climate change of Adele Penguins under Dr. Steven Emslie. In 2005, he entered the graduate program in Marine Biology at the University of North Carolina Wilmington where he worked under the guidance of Dr. Richard Satterlie. Mr. Hayward graduated in December 2007.

APPENDIX

A. Interpulse interval (IPI) data from experimental animals. The intervals were separated into 250 msec bins. Mean number of contractions for tow rhopalia trials (*Aurelia aurita*, n=6)
B. Interpulse interval (IPI) data from experimental animals. The intervals were separated into 250 msec bins. Mean number of contractions for three rhopalia trials (*Aurelia aurita*, n=6)
C. Interpulse interval (IPI) data from experimental animals. The intervals were separated into 250 msec bins. Mean number of contractions for four rhopalia trials (*Aurelia aurita*, n=6)
D. Interpulse interval (IPI) data from experimental animals. The intervals were separated into 250 msec bins. Mean number of contractions for five rhopalia trials (*Aurelia aurita*, n=7)
E. Interpulse interval (IPI) data from experimental animals. The intervals were separated into 250 msec bins. Mean number of contractions for six rhopalia trials (*Aurelia aurita*, n=8)
F. Interpulse interval (IPI) data from experimental animals. The intervals were separated into 250 msec bins. Mean number of contractions for seven rhopalia trials (*Aurelia aurita*, n=10)

INTRODUCTION

Overview

Cnidarians are simple multicellular marine organisms constructed at the tissue level of organization. Common dogma suggests they have the simplest multicellular nervous systems in the animal kingdom. The generalized cnidarian nervous system includes diffuse, non-polarized nerve nets made up of bipolar and multipolar neurons that communicate via chemical synapses (all classes) and gap junctions (hydrozoans; Satterlie, 2002). Despite this perceived neurobiological simplicity, the behavioral repertoires of cnidarians can be rich.

Scyphomedusae are weak swimmers and are at the mercy of prevailing currents and wind-driven surface waves (Eiane et al., 1999). They normally are found at or near the surface, and are often beached. This results in sporadic, high-density jellyfish blooms during the summer months. Most species of scyphomedusae are able to deliver painful stings that can potentially cause serious health problems in sensitive individuals. Due to their potential ecological significance, and their threat to public health, we need a full understanding of their behavioral physiology.

Statement of Problem

Scyphozoans normally have a minimum of eight rhopalia, although some species have sixteen while others have as many as sixty-four (Satterlie, 2002); this is important since each rhopalium contains a swim pacemaker in addition to sensory structures. The physiology of pacemakers presents an interesting question about the functional significance of this apparent pacemaker redundancy, from the point of view of control of the swim musculature. Previous

studies have shown that individual pacemakers have an irregular output (Lerner et al., 1971; Murray, 1977; Satterlie and Nolen, 2001).

Frequency-dependent facilitation occurs when a pacemaker fires, this contraction leaves behind an altered state so that the next contraction is enhanced. A change in the frequency of stimuli during these series of contractions results in an immediate change in contraction strength (Bullock, 1943). Since frequency-dependent facilitation is an important determinant of muscle force output in scyphozoans (Bullock, 1943; Passano, 1965; Satterlie, 2002), an irregular pacemaker output may not produce adequate drive to the swim muscles to keep the animals in the water column (Lerner et al., 1971; Murray, 1977; Satterlie and Nolen, 2001). However, linking multiple pacemakers in scyphomedusae and cubomedusae causes an overall increase in the frequency and regularity of swim contractions, which provides for efficient locomotion (Lerner et al., 1971; Murray, 1977; Satterlie and Nolen, 2001). Thus, the redundancy of pacemakers serves an adaptive function only if fast and regular swimming contractions are more useful to the jellyfish than slow and irregular swimming contractions (Lerner et al., 1971). Here, the investigation of pacemaker redundancy is take one-step further, to test the nature of the connections between pacemakers in three different species of scyphozoan jellyfish, and in the ephyra of one of the species.

The simplest model to explain pacemaker interactions in producing this faster, more regular rhythm is that of a network of resetting pacemakers in which one pacemaker triggers a contraction wave in the swim musculature and resets all of the other pacemakers (Lerner et al., 1971; Murray, 1977). In this way, the pacemaker with the fastest rhythm will drive swimming until its output frequency drops and another pacemaker takes over. An alternate model suggests that a regular rhythm will also emerge if there is no resetting of pacemakers, and their output is

totally independent (Satterlie and Nolen, 2001). In modeling studies of cubozoan jellyfish, Satterlie and Nolen (2001) found the pacemaker networks fell between the resetting and independent models, suggesting they are semi-independent; pacemakers are capable of escaping from a strict resetting organization through the influence of various inputs to the individual pacemakers. In keeping with past modeling results on scyphozoan jellyfish (Horridge, 1959; Lerner et al., 1971; Murray, 1977), I predict that regular swim activity in these rather sluggish swimmers is due to the redundancy of pacemakers in pacemaker networks that have strict resetting connections.

Location and Coordination of Pacemakers

To aid in swimming, jellyfish have both radial muscle (runs toward the bell apex) and circular muscle (ring-like organization around the bell; Horridge, 1954b; fig. 1A). Swimming is achieved by ejecting water from the opening of the bell by contraction of circular muscles, and in some cases, radial muscles (Satterlie and Spencer, 1987). Circular muscle cells are located deep within the subumbrella, are striated, and range from 0.2-0.3 microns in diameter (Gladfelter, 1972; Satterlie and Spencer, 1979). Scyphozoan contractions are relatively slow, with contraction and relaxation sometimes taking as long as two seconds (Bullock, 1943). Scyphomedusae swim through the symmetrical contraction of the circular muscles, while asymmetrical contractions are used for turning (Horridge, 1959). In addition, several species of scyphomedusae use swimming to generate eddies that deliver and trap prey in the marginal tentacles (Matanoski et al., 2001). Thus, swimming is used to obtain prey and to maintain the animal's position in an area where prey are found (Matanoski et al., 2001; Matanoski and Hood, 2006). Interestingly, as prey density increased, *Chrysaora quinquecirrha*'s swim frequency

increased, and when prey density decreased its swimming frequency decreased (Ford et al. 1997; Matanoski et al., 2001). Thus, *C. quinquecirrha* may have some degree of prey-selected control over swim contractions.

True to their radial symmetry, rhopalia are found around the margin of the bell of scyphozoans, oriented to allow gathering of sensory information from all directions without a dedicated leading body part (Horridge, 1954b; Satterlie, 2002; Romanes, 1876). Each rhopalium includes a statocyst for determining orientation, pigmented light sensitive eyespots (ocelli), and sensory pits that are believed to be chemoreceptive. Within each rhopalium is a "marginal nerve center" (pacemaker) that serves to set the frequency of swim contractions (Horridge, 1954; Lerner et al., 1971; Romanes, 1876). Pacemakers are groups of cells that possess the ability to generate spontaneous action potentials (Horridge, 1959). Each individual action potential from a pacemaker, in turn, generates a single swim contraction of the subumbrellar musculature. Local sensory information is presumably integrated within a rhopalium, which can lead to changes in the frequency characteristics of its pacemaker output (Horridge, 1959). Isolated rhopalia are capable of generating impulses independently; therefore, the animal is presumed to have some means of pacemaker coordination (Murray, 1977; Romanes, 1876; Satterlie and Spencer, 1987).

The removal of a rhopalium does not alter the conduction of impulses through the muscle layer (Romanes, 1876; Satterlie, 2002). Due to the redundancy of pacemakers, swimming will continue in spite of serious damage to the nerve nets (Lerner et al., 1971). With the large number of rhopalia found in scyphomedusa, there is a constant shift of control between each of the rhopalia (Horridge 1956; Lerner et al., 1971; Murray, 1977). This shift is influenced by sensory inputs since in an artificially tilted animal; contractions usually originate from the upper-most rhopalium. However, when that rhopalium is brought to the lowest position, that particular

rhopalium no longer drives swimming (Horridge, 1956). Past modeling results suggest the activity from an active pacemaker resets all others (resetting pacemaker network), giving rise to the concept that the pacemaker with the fastest rhythm controls swim output (Horridge, 1959; Lerner et al., 1971; Murray, 1977).

Romanes (1876) discovered that scyphomedusae have two types of nerve nets: the motor nerve net (MNN; fig. 1A) and the diffuse nerve net (DNN; fig. 1B). Both the MNN and the DNN have symmetrical chemical synapses so each neuron can serve as the presynaptic or postsynaptic cell. These features allow the nerve nets to be nonpolarized so a contraction will be conducted throughout the entire subumbrella, in any direction, regardless of the site of initiation (Horridge, 1954b; Satterlie, 2002). The DNN can influence pacemaker output by indirectly stimulating the acceleration of the swim rhythm; in addition, it provides peripheral modulatory input directly to the swim musculature, allowing a wide range of contraction patterns (Satterlie, 2002).

The Motor Nerve Net

The motor nerve net extends throughout the entire subumbrella, forming a network that connects all eight marginal rhopalia with the circular and radial swimming muscle bands (Carlberg et al., 1995). In addition, the network has direct neuromuscular synapses that activate the swim musculature. As mentioned above, two important properties underline the simplicity and important functional properties of the MNN: (1) impulses generated in one part of the conduction system are transmitted through the entire net, so the spread of excitation is diffuse, and (2) excitation can occur in any direction throughout the nerve net (Satterlie, 2002).

A single impulse from a rhopalium results in an excitation wave that is conducted throughout the entire MNN at about 50 cm/sec (Satterlie and Spencer, 1987). The relatively slow kinetics of the swim contraction produces a symmetrical contraction of the bell (Passano, 1965). The MNN has a refractory period that is 200-250 milliseconds long (Bullock and Horridge, 1965), thus limiting the possibility of tetanic contractions of the subumbrellar musculature. The refractory period decreases in duration with repetitive activity, though suggesting there is some plasticity related to high frequency swim activity (Bullock and Horridge, 1965). There are reports of parallel increases in conduction velocity within the MNN following repetitive activity (see Bullock and Horridge, 1965).

Neurons found within the MNN are bipolar or multipolar and have cell body diameters of around 15μm (Anderson and Schwab, 1983). Individual neurons have stable resting potentials (-60 to -70 mV) and action potentials with 10 – 20 msec durations (Anderson and Schwab, 1982).

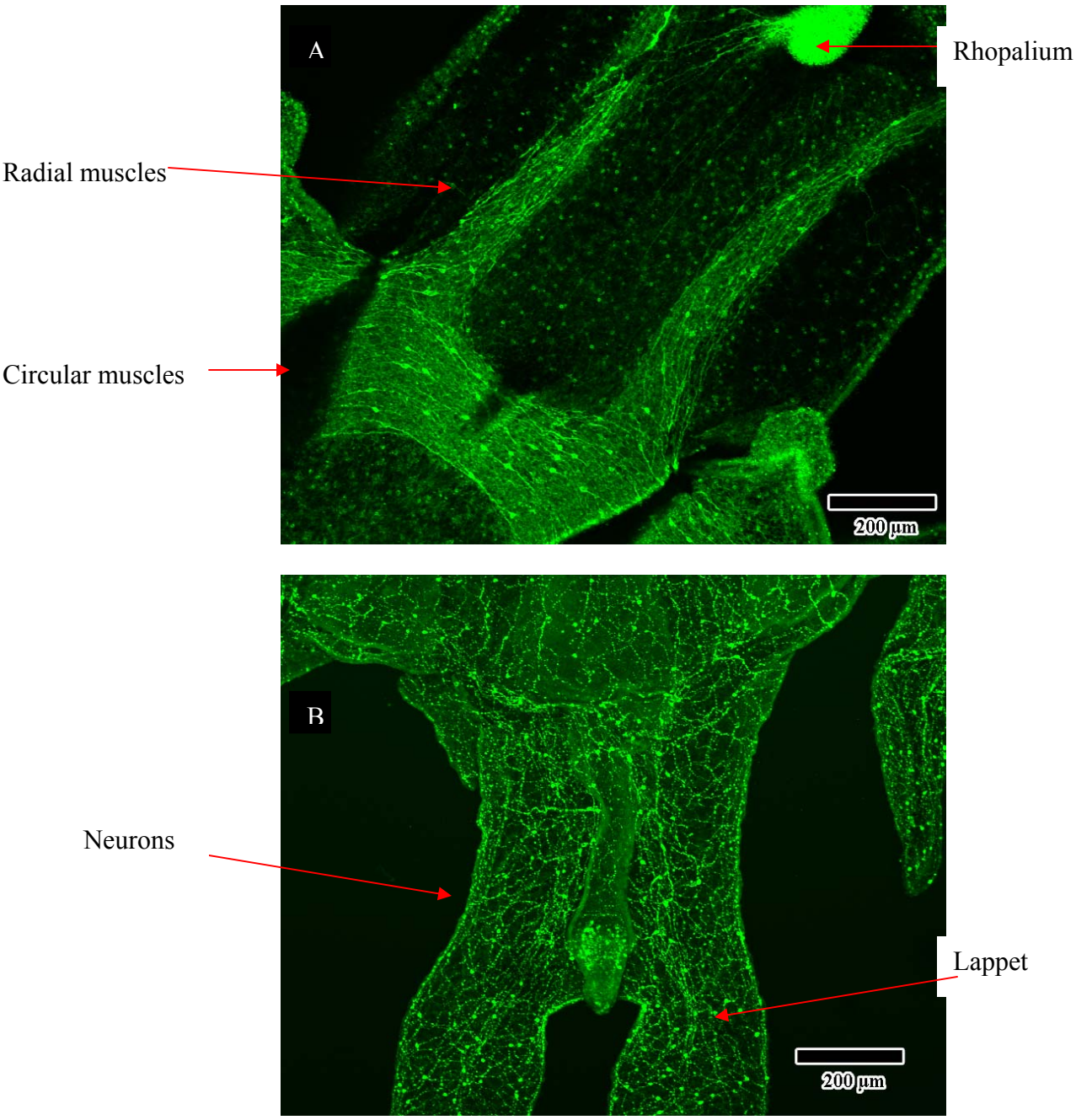

Figure 1. Fluorescence micrograph of the motor nerve net (MNN) of *Aurelia aurita*, using immunohistochemistry labeling of an antibody to Tubulin. (A) Detailed picture of the motor nerve net of the subumbrella swim muscles. Depicts the neurons running from the rhopalium to the motor nerve net. (B) Diffuse nerve net (DNN) of *Aurelia aurita*, using immunohistochemistry labeling an antibody to FMRF.

The Diffuse Nerve Net

The diffuse nerve net (DNN) of scyphomedusae is found throughout the subumbrella, exumbrella, manubrium, tentacles, and oral arms. This system aids in the coordination of localized movements such as feeding, passes impulses relatively slowly, and is capable of altering the firing activity of pacemakers (Passano, 1965).

Conduction velocity in the DNN is slow (in the 15cm/s range), variable, and subject to facilitation (Bullock and Horridge, 1965). Even though the conduction of impulses occurs across the subumbrella, the DNN cannot directly produce a contraction of the swim musculature, although they can up-modulate ongoing swimming activity. The only point of interaction between the DNN and the MNN is in the rhopalia, through DNN-induced changes in pacemaker output. Contractions of the DNN can cause tentacle shortening, acceleration of swim beats, and contractions of the manubrium (Bullock and Horridge, 1965).

Pacemaker Activity

The activity of a single pacemaker is irregular; however, by linking multiple irregular pacemakers the swim system exhibits regular contractions (Lerner et al., 1971; Murray, 1977; Satterlie and Nolen, 2001). Thus, multiple pacemakers increase the frequency and regularity of swim contractions (Horridge, 1954a, 1954b, 1956; Lerner et al., 1971; Murray, 1977; Romanes, 1876). In addition, as the numbers of pacemakers are increased, the sensitivity to sensory inputs (disturbances) increases. There is a constant shift of control between the rhopalia, which may explain why scyphomedusae have such a large number of rhopalia (Murray, 1977; Satterlie, 2002).

What prevents the system becoming chaotic? This is believed to be dependent on the resetting response from discharging pacemakers (Pantin and Dias, 1952; Murray, 1977). If more than one pacemaker discharges at a time two separate contraction waves are initiated in the subumbrella. When the two-waves collide, they cancel each other at the point of collision due to "mutual refractoriness" (Satterlie, 2002). Even in these cases like this, only a single, symmetrical contraction of the subumbrella is produced (Murray, 1977; Satterlie 2002). This raises the possibility that a network of totally independent pacemakers could still produce a regular, efficient output within the swimming system.

Other studies have reveled affects that are not consistent with a simple resetting mechanism (Horridge, 1959; Satterlie and Nolen, 2001). Satterlie and Nolen suggested three possibilities for constructing pacemaker networks: independent, resetting, or a semi-independent. An independent neural network consists of pacemakers that do not directly interact with one another. As mentioned previously, in a resetting pacemaker network, activity in one pacemaker will reset all of the other pacemakers and thus the pacemaker with the fastest rhythm will control the swim contractions (Murray, 1977). A semi-independent neural network is one that shows full resetting connections, but allows modulation of the resetting connections so that they become temporarily independent, such as though sensory or modulatory inputs. Thus, individual pacemakers may be under the resetting influence at times (resetting) or free from the resetting influence at other times (independent).

Potential Contributions and Limitations of the Study

The main objectives of this project are to (1) understand the swimming behavior of three species of scyphozoan jellyfish, by investigating of the role of pacemaker number on swimming

frequency, regularity of swimming, and (2) identify whether these species posses have resetting, independent, or semi-independent pacemaker networks. Modeling experiments were conducted on pacemaker interactions in *Chrysaora quinquecirrha*, *Stomolophus meleagris*, *Aurelia aurita*, and the ephyra of *Aurelia aurita* (to give a developmental perspective). In the modeling experiments, artificial pacemaker networks were based on the data from single pacemaker preparations, with internal sources of variation included so that individual model pacemakers produced similar interpulse intervals (IPI). These pacemakers were then either connected into resetting networks or allowed to free run without any inter-pacemaker interactions. In addition, the models allowed insertion of variable levels of partial resetting.

Due to the high number of pacemakers in scyphomedusae, and the existing modeling results from previous work (Horridge, 1959; Lerner et al., 1971; Murray, 1979), I predict that pacemaker networks of these three species will be biased toward shorter interpulse intervals and will be of the resetting type. In a similar investigation showed that the swim pacemaker network of cubomedusae fell between the resetting and independent models, suggesting a semi-independence of swim pacemakers (Satterlie and Nolen, 2001). Cubomedusae have only four swim pacemakers (four rhopalia), and the combination of the small pacemaker number and semi-independent connections allowed a great biphasic modulatory potential for these active swimmers. I suggest the higher pacemaker number of scyphomedusae, and their relatively sluggish swimming and turning abilities, may not reflect a pacemaker network organization like that of cubomedusae.

MATERIALS AND METHODS

Pacemaker interactions in *Chrysaora quinquecirrha* (Desor, 1848), *Stomolophus meleagris* (L. Agassiz, 1862), *Aurelia aurita* (Linnaeus, 1758), and the ephyra of *Aurelia aurita* were investigated using artificial pacemaker network models created with data from pacemaker ablation experiments. Models outputs were then compared to actual data from experiments. A total of 145 scyphozoan jellyfish were used: 59 *Aurelia aurita*, 40 ephyra of *Aurelia aurita*, 22 *Chrysaora quinquecirrha*, and 24 *Stomolophus meleagris*. *C. quinquecirrha* and *S. meleagris* were collected using dip nets, by hand, or bottom trawls. Some individuals of *C. quinquecirrha* came from a culture located at the North Carolina Aquarium at Fort Fisher. All adult *A. aurita* and its ephyra came from a year round culture at the Center for Marine Science, Wilmington, NC. Jellyfish sizes ranged from 5 mm bell diameter (ephyra) to 15 cm bell diameter. All observations were made on healthy jellyfish whose pacemakers were in good condition.

A 10-gallon aquarium was filled with filtered seawater and one jellyfish was put into the aquarium for 1 hour to acclimate. Acclimation was considered successful if the medusa exhibited constant swimming beats regardless of number of rhopalia, and no cyclical changes in swimming. In similar experiments by Romanes (1877) and Horridge (1959), the swimming rate became constant about 30 minutes after removal and remained so for several hours.

A data trial involved measurement of interpulse intervals (IPI's), separated into 250 millisecond bins. The data were presented as frequency histograms. Before each trial, the bell diameter, number of current rhopalia, and place and method of capture was recorded for each jellyfish. Due to the morphological characteristics (rigidity of the bell) of *S. meleagris*, bell length as well as bell diameter was recorded.

Ablation Techniques

A single trial consisted of ablating an appropriate number of rhopalia prior to data collection. Ablation randomization was accomplished by rolling an 8-sided die to determine which rhopalia would be removed (each species has 8 rhopalia). Rhopalia were ablated by cutting small pie-shaped pieces from the margin of the bell using scissors. The jellyfish was then orientated with the number 1 rhopalium at the 12 o'clock position, number 3 in the 3 o'clock position, number 5 in the 6 o'clock position, and number 7 in the 9 o'clock position (fig. 2). The number of rhopalia that were ablated was based on the number of rhopalia needed for each trial (i.e. trial consisting of 3 rhopalia then 5 rhopalia were ablated).

Trials lasted up to 1,000 swim contractions or for one hour (depending on which one came first). A computerized program "event timer" developed by Jason Richardson was used to record swim contractions, with each contraction registered by the push of the computer spacebar. The event timer logged the number of contractions, the time between each contraction (IPI), the length of the trial, and put each interpulse interval into appropriate 250-millisecond bins. Individual *Aurelia aurita* and the ephyra of *Aurelia aurita* were used once in each trial. Due to the limitations in collecting *C. quinquecirrha* and *S. meleagris*, individuals were used for two trials each. In these particular cases, the initial jellyfish trials started with 8 rhopalia and after the initial trial, either 1, 2, 3, 4, 5, 6, or 7 rhopalium/rhopalia were ablated. Therefore, all possible numbers of pacemakers were removed.

Data were incorporated into artificial pacemaker models developed by Dr. Thomas Nolen, of State University of New York, at Suny New Paltz. The models create species-specific artificial pacemaker networks that allowed comparison of the pacemaker networks of the three scyphomedusae species, and allowed testing whether the networks had resetting, independent, or

semi-independent pacemaker interactions. By Models of individual pacemakers were based on the ablation trials from single pacemaker animals of the appropriate species. Identical, individual model pacemakers were then linked in a resetting pacemaker network, or allowed to free run without interactions with other pacemakers (independent network). The models produced IPI data just like the experimental animals. Due to the high number of pacemakers in scyphomedusae, it was predicted that the neural networks of these three species would be biased toward shorter interpulse intervals and have resetting pacemaker networks.

Integrate and fire pacemaker model

The integrate and fire model is the simplest model of a spiking neuron that takes into account the dynamics of its input (Dayan and Abbott, 2001; fig. 3). The basis of the integrate-and-fire model is a simple compartmental model of a neuron: where I_{in} is the input current (i.e., from a synapse), C is the membrane capacitance, R is the net membrane resistance due to all passive channels, and V_{rest} is the resting potential of the neuron (typically about -70 mV; fig. 3). Thus, if no current is injected ($I_{in} = 0$) and the

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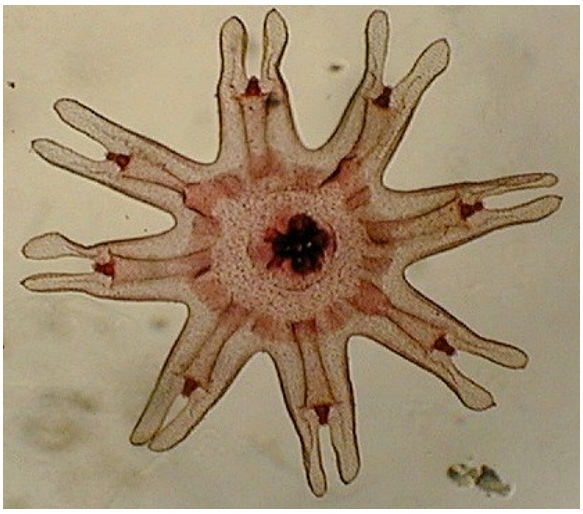

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Figure 2. Orientation the ephyra of *A. aurita* just before ablation of its rhopalium/rhopalia; this orientation was used for all of the jellyfish in the study.

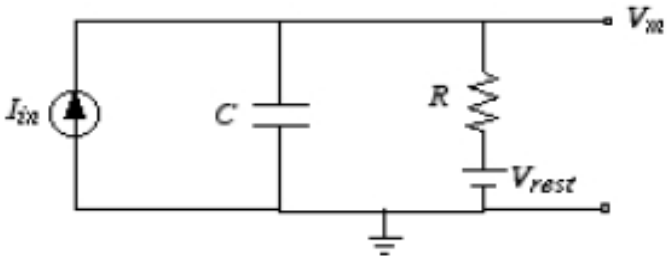


Figure 3. The basis of the integrate and fire model is the simple compartmental model of a neuron.

system has come to equilibrium then the membrane potential (Vm), will equal the resting potential, V_{rest}.

In the model, each integrate and fire pacemaker has a membrane potential (V_m) that varies between rest and threshold, and has a probability of firing that varies between 0 and 1. For a single pacemaker, the probability of firing, P, is given by: $P = 1 – (Th – V_m)$, where V_m is the membrane potential and Th is the threshold for firing. (Thus, the probability of firing is determined by how close V_m is to Th). V_m varies as a function of (random) synaptic input, both excitatory and inhibitory. So, for a single pacemaker, its membrane potential, V_m at time t is given by: $V_m = V_0 + \xi e(t) – \xi i(t)$. Where V_0 represents the membrane potential at t-1, $\xi e(t)$ and $\xi i(t)$ are white noise sources of synaptic excitation and inhibition. When P approaches one, an action potential is recorded and V_m is reset to resting.

For a network pacemaker receiving inhibitory inputs from N pacemakers, the probability that the i^{th} pacemaker will fire is: $P_i = 1 – (Th – V_i)$, where V_i is the membrane potential of the ith pacemaker, and Th is that pacemaker's threshold. P_i varies between 0 and 1; when P_i goes to one, an action potential is propagated around the nerve net and the ith pacemaker is reset to rest. The i^{th} pacemaker's membrane potential, V_m at time t is given by: $V_m = V_0 + \xi e(t) – \xi i(t) – \Sigma \delta ij(t')$, where V_0 represents the membrane potential at t-1, $\xi e(t)$ and $\xi i(t)$ are white noise sources of synaptic excitation and inhibition, $\Sigma \delta ij(t')$ is the sum of synaptic inputs from the other N pacemakers. Finally, $\delta ij(t') = s\alpha^2 t' e^{-\alpha t'}$ where s and α indicate the strength and duration of a postsynaptic potential, and t' is the time after the jth pacemaker fired.

The integrate and fire pacemaker model provides a pacemaker output and a network output stochastically, simulating the time varying V_m of hypothetical pacemakers using a Do Loop using Igor Pro 6.0 software (Wavemeterics Inc). Model parameters such as threshold,

synaptic noise and refractory period were changed, as were pacemaker interactions, such as resetting, via the $\Sigma \delta ij(t')$ term. Parameters (particularly the noise function) were changed to adjust the mean interpulse interval (IPI) for a model pacemaker to be close to that of real jellyfish/single rhopalium data. The mean, standard deviation (S.D.) of the IPI distribution, and the ratio of S.D. to mean, called the coefficient of variation (CV= S.D./mean IPI) were calculated.

The degree of resetting by pacemakers was varied (0%, 50%, and 100%) using the intergrate and pacemaker model. The model networks were run until there were 1,000 IPIs and 1 trial was carried out for each network. Means and S.D. of the IPI distribution parameters were calculated for each trial. For comparisons with the real animal data, average values for each of the model distribution parameters were plotted (IPI Mean, S.D., CV) +/- 1 standard deviation.

Model networks were programmed with Igor Pro 6.0 software (Wavemeterics Inc, Lake Oswego, Oregon). Each network consisted of individual oscillators running together and each oscillator was designed to fire with an output that cycled from baseline to threshold with a constant slope. To add variability, random increments and decrements were added to the probability; therefore, when the probability reached threshold, a pacemaker impulse was generated. Igor Pro 6.0 produced noise generators that were reseeded for each iteration and produced a Gaussian output. For each experimental run, the model sampled between 800 and 1000 IPIs and provided an appropriate IPI distribution. Each time the model was run for each set of characteristics, the IPI distribution was similar; however, the mean and standard deviations were different. Thus, the model was able to produce consistency in the IPI distributions even though the raw data were different in each run.

Statistical Analysis

Linear regression of the coefficient of variation (CV) of the interpulse intervals versus the number of pacemakers was calculated and plotted for the different models (0% resetting, 50% resetting, and 100% resetting) and animals. Pairwise comparisons of the animals interpulse interval with the three models: independent (0% resetting), 50% resetting, and 100% resetting, were made using a t-test.

RESULTS

Swim behavior was similar for all three species of jellyfish *Aurelia*, *Chrysaora*, and *Stomolophus*, and for the ephyra of *Aurelia*. A considerable difference in buoyancy was noted however. Both *Aurelia* and *Chrysaora* were near neutral buoyancy, so they remained in the water column if swim frequency decreased or stopped for short periods of time. *Stomolophus*, and the *Aurelia* ephyra, were negatively buoyant, and sank to the bottom of the aquarium if swimming was interrupted for even brief periods.

In all medusae examined, removal of rhopalia decreased the overall frequency and regularity of swimming (see appendix). These changes were observed as increases in the means and standard deviations of interpulse intervals (IPIs) with decreases in rhopalial number. If all rhopalia were removed, the swim systems were paralyzed, with only occasional, weak contractions of the circular musculature, with one exception. *Stomolophus* (the cannonball jellyfish) had an extremely fast swimming rhythm, and after removal of all rhopalia, coordinated swim contractions continued, but contractions were not strong enough to lift the animals from the bottom of the aquarium. This presents an interesting contrast to the other species, both in the location of pacemakers and in the overall excitability of the swim systems. *Stomolophus* has more of a rounded shape, with a solid mass of oral arms that hang well below the opening of the bell (which is round in shape instead of disc shaped, like the other species). In addition, it is much more "solid", in terms of tissue density, than the other species, perhaps producing this negative buoyancy. On casual observation, the swim contractions of *Stomolophus* were highly efficient at ejecting water from the bell, compared to *Aurelia* and *Chrysaora* (based on impact of each contraction on movements of the bell). It is possible the high frequency

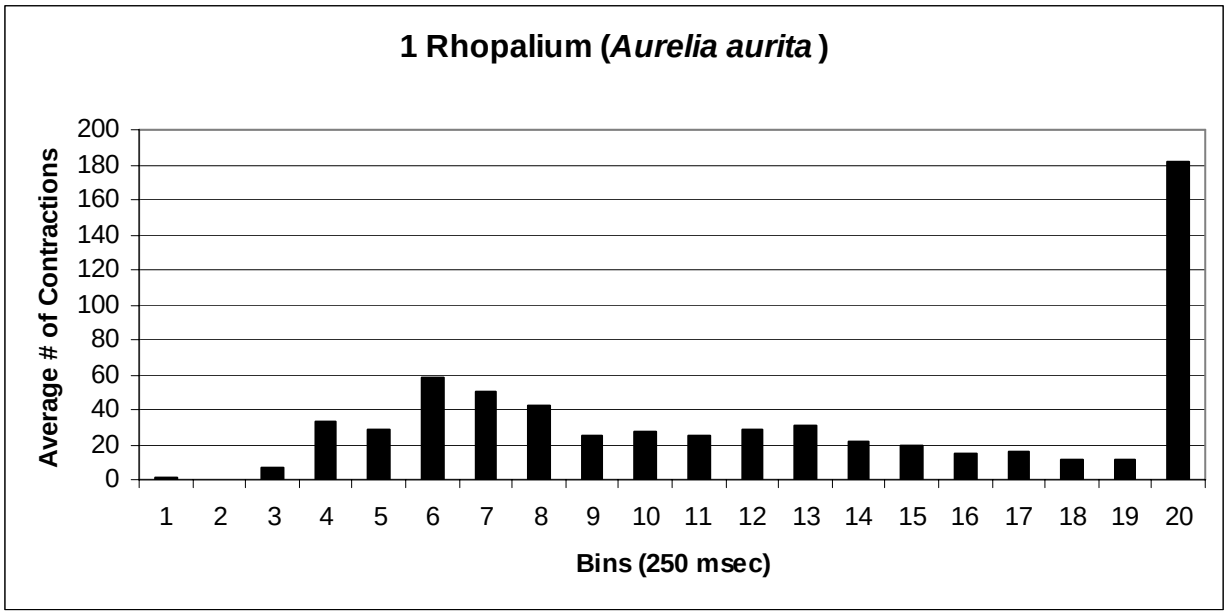


Figure 4. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 ms bins. Mean number of contractions for one rhopalium trials (*Aurelia aurita*, n=11). Note that bin 20 = >4,750 msec.

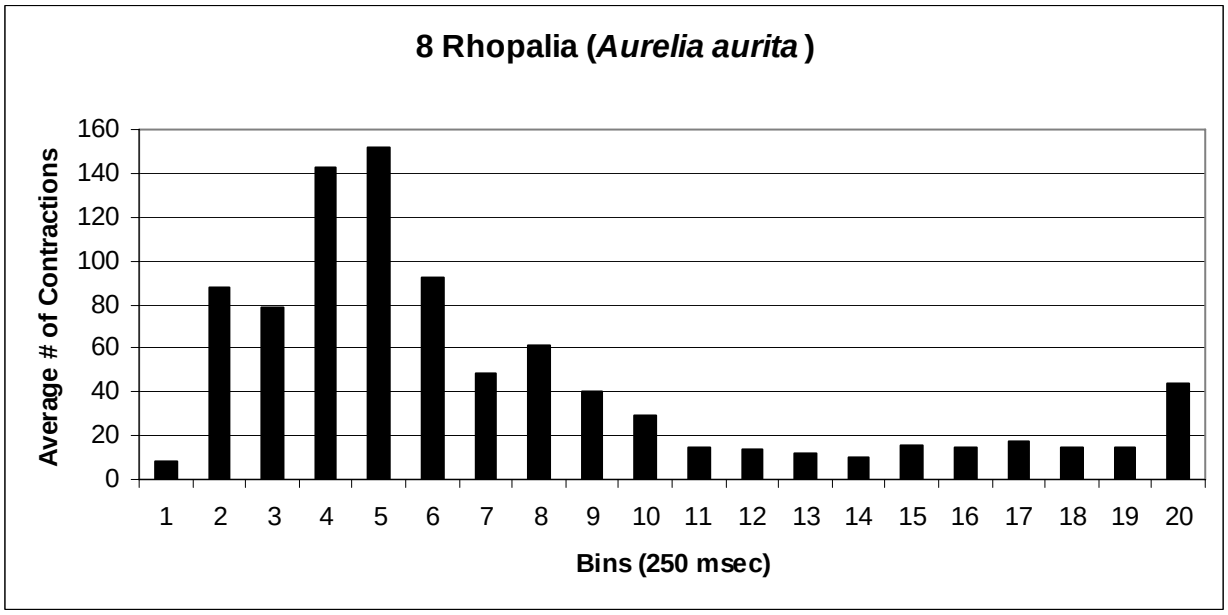


Figure 5. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 ms bins. Mean number of contractions for eight rhopalia trials (*Aurelia aurita*, n=9). As the number of pacemakers increase, the distribution of interpulse intervals moves towards shorter intervals.

contractions and the greater excitability of the swim system of *Stomolophus* are necessary to keep this more "solid" medusa in the water column.

Aurelia aurita (adult medusae)

Interpulse interval profiles produced by single rhopalium preparations had long tails and a high IPI variation (fig 4). When pacemakers were added to the network, the mean IPI and the regularity of swimming increased, and the distribution of interpulse intervals moved toward shorter intervals. For example, single pacemaker preparations swam with a mean IPI of 5530 msec +/- 2530 msec S.D., while the 8-rhopalium preparations had a mean IPI 1880 msec +/- 1180 msec S.D. (fig. 12A). This is consistent with the modeling results of Horridge (1959), Lerner et al. (1971), and Murray (1977) that suggested that long interpulse intervals are less frequent when many pacemakers are present.

In integrate and fire models of pacemaker networks constructed from artificial pacemakers with IPI profiles identical to real *Aurelia* single-pacemaker preparations, addition of pacemakers also decreased mean IPI and reduced variance, regardless of the type of pacemaker network simulated (resetting or independent). Comparison of real data for one through eight pacemaker preparations to both resetting and independent model networks showed that the real data were closer to the resetting model. However, the real data were intermediate between the two models (fig. 12; table 1). Only the 50% resetting model (semi independent) was not statistically significantly different from *Aurelia* (table 1; $P>0.05$). In contrast, the other models were significantly different from the real animal networks. The best fit of real data to modeled data was found with a 50%

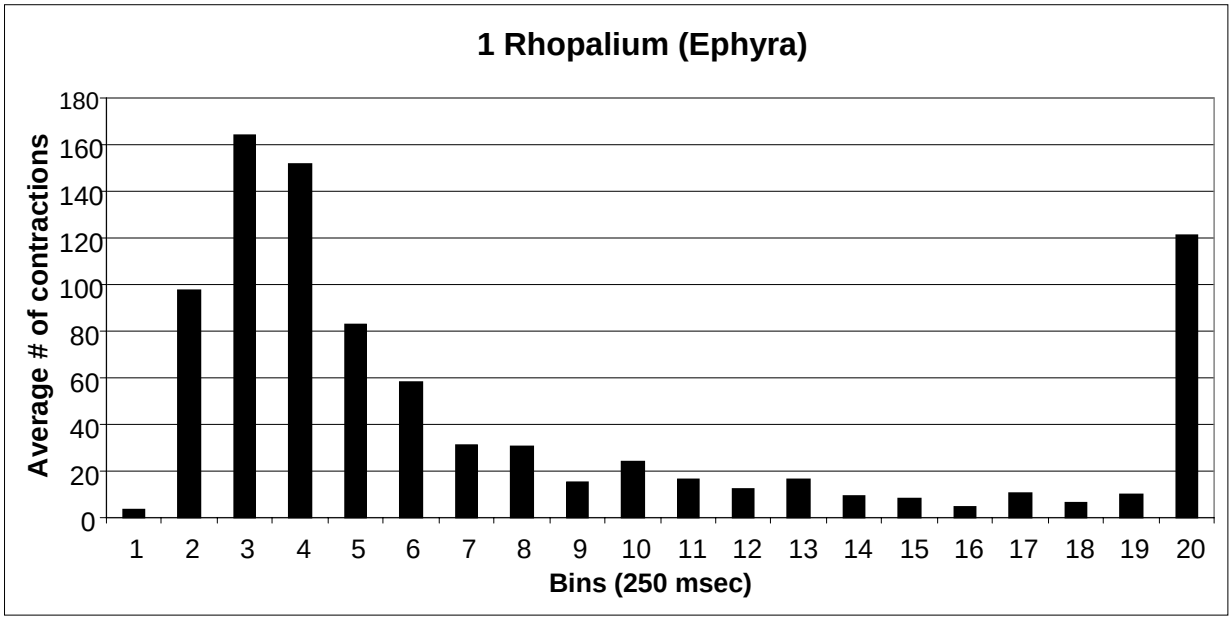


Figure 6. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins Mean number of contractions for one rhopalium trials (*Aurelia aurita* ephyra, n=5). Note that bin 20 = >4,750 msec.

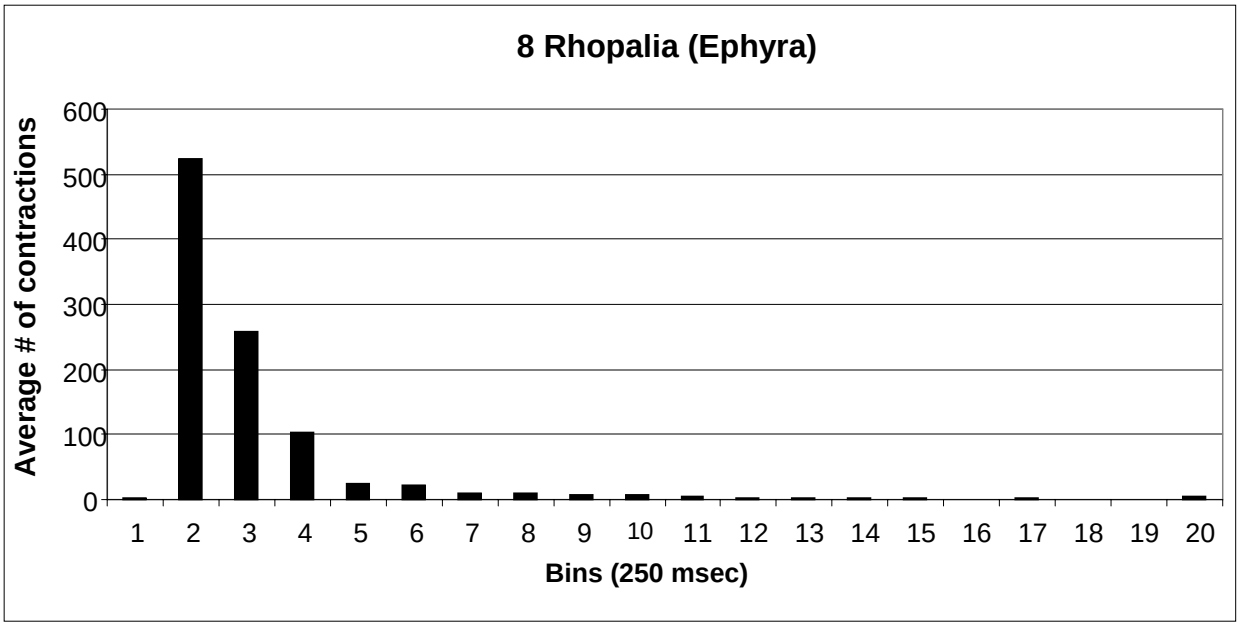


Figure 7. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for eight rhopalia trials (*Aurelia aurita* ephyra, n=5). As the number of pacemakers increase, the distribution of interpulse intervals moves towards shorter intervals.

resetting network, suggesting the pacemaker networks in adult *Aurelia* are semi-independent—the pacemakers show some degree of independence, but with a degree of resetting as well.

Aurelia aurita (ephyra)

As with adult medusae, preparations with larger numbers of pacemakers had shorter mean IPIs, with less variance suggesting a positive correlation between rhopalium number and swim regularity and frequency (figs 6, 7). Single pacemaker preparations had a mean IPI of 3620 msec +/- 3320 S.D. while animals with all eight rhopalia had a mean IPI of 690 msec +/- 24 msec S.D. (fig. 13A).

A comparison of real ephyra data to resetting, independent, and 50% resetting model networks (based on pacemakers with IPI profiles similar to those of single-rhopalium preparations) gave a best fit with the model of the 50% resetting and 100% resetting network (fig. 13C).

The 50% resetting model (semi independent) and the 100% resetting model were not statistically significantly different from the ephyra data (table 1; $P>0.05$). In contrast, the independent model (0% resetting) was significantly different from the data. Thus, either the resetting or the semi-independent models can satisfactorily explain the ephyra network, although the resetting network gives the better fit (fig. 14; table 1).

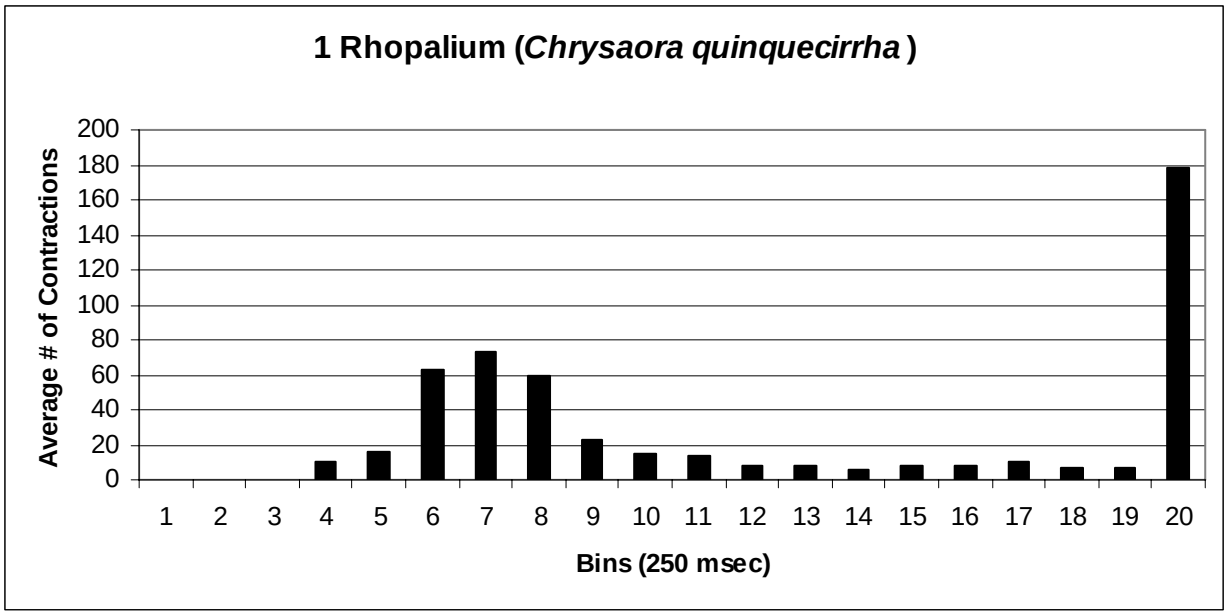


Figure 8. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for one rhopalium trials (*Chrysaora quinquecirrha*, n=3). Note that bin 20 = >4,750 msec.

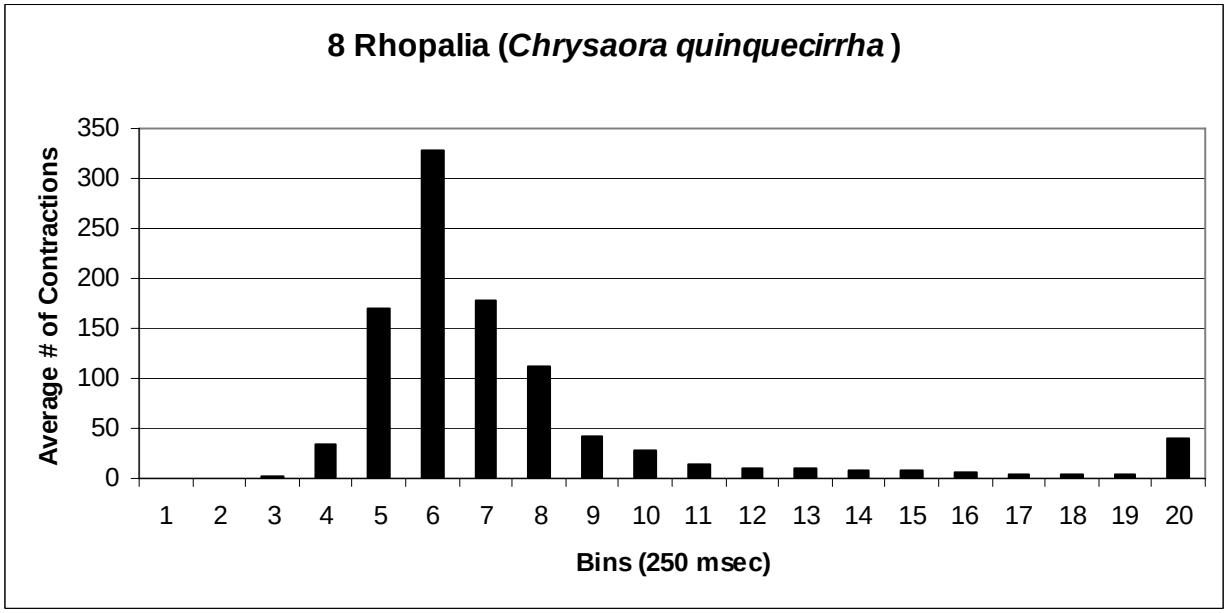


Figure 9. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for eight rhopalia trials (*Chrysaora quinquecirrha*, n=20). As the number of pacemakers increase, the distribution of interpulse intervals moves towards shorter intervals.

Chrysaora quinquecirrha

Similar to *Aurelia*, addition of pacemakers to the swim system increased the frequency and regularity of swimming (figs 8, 9). Single pacemaker preparations had a mean IPI of 11100 msec +/- 8400 msec S.D., while intact animals (all 8 rhopalia) had an IPI of 1930 msec +/- 810 msec S.D. Thus, in *Chrysaora*, the influence of rhopalial number on the regularity and frequency of swim contractions was dramatic.

A comparison of real data with resetting, 50% resetting and independent models (constructed with pacemakers with IPI profiles similar to *Chrysaora* single-rhopalium preparations), indicated the pacemaker networks in the real animals are of the resetting type (fig. 15). The experimental data showed a best fit with models exhibiting 100% resetting interactions. When the data was compared to the independent network and the 50% resetting network. The independent network was extremely significant ($P<0.001$) while the 50% resetting model was very significant ($P<0.01$). In contrast, the 100% resetting model was not significantly different ($P>0.05$) when it was compared to the animal data (table 1).

Stomolophus meleagris

Even though *Stomolophus* preparations were much more excitable (higher frequency contractions in intact animals and in all ablation experiments), a positive correlation between pacemaker number and frequency and regularity of swimming was still evident (figs 10, 11). In single peacemaker preparations, the mean IPI was 860 msec +/- 400 msec S.D., while in intact medusae, the mean IPI was 710 msec +/- 190 msec

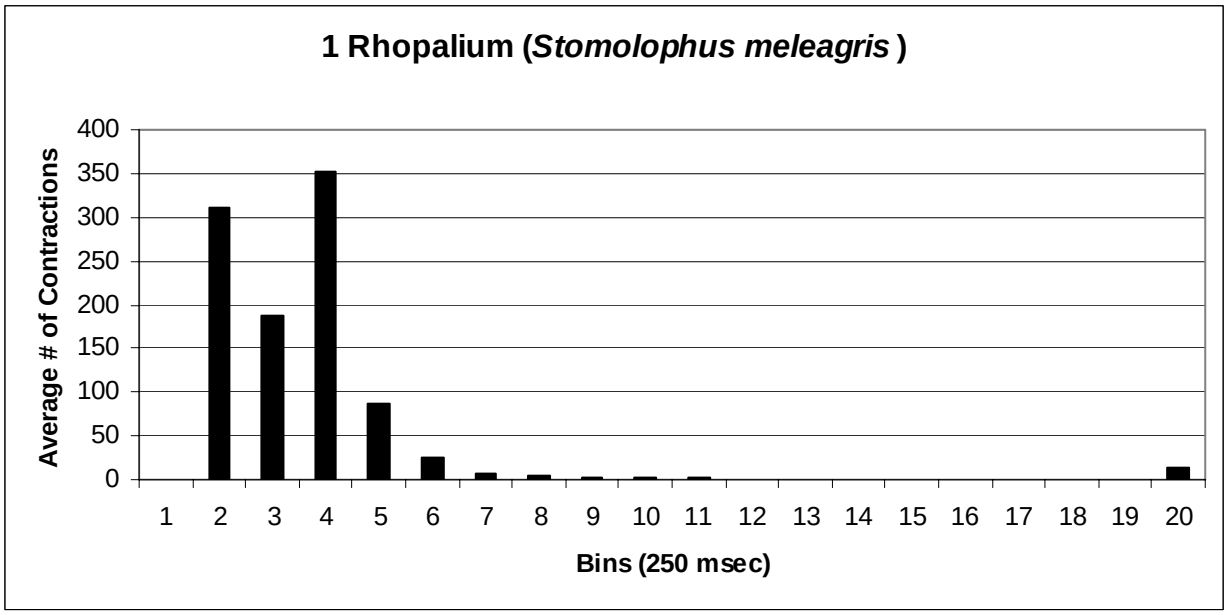


Figure 10. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for one rhopalium trials (*Stomolophus meleagris*, n=6). Note that bin 20 = >4,750 msec.

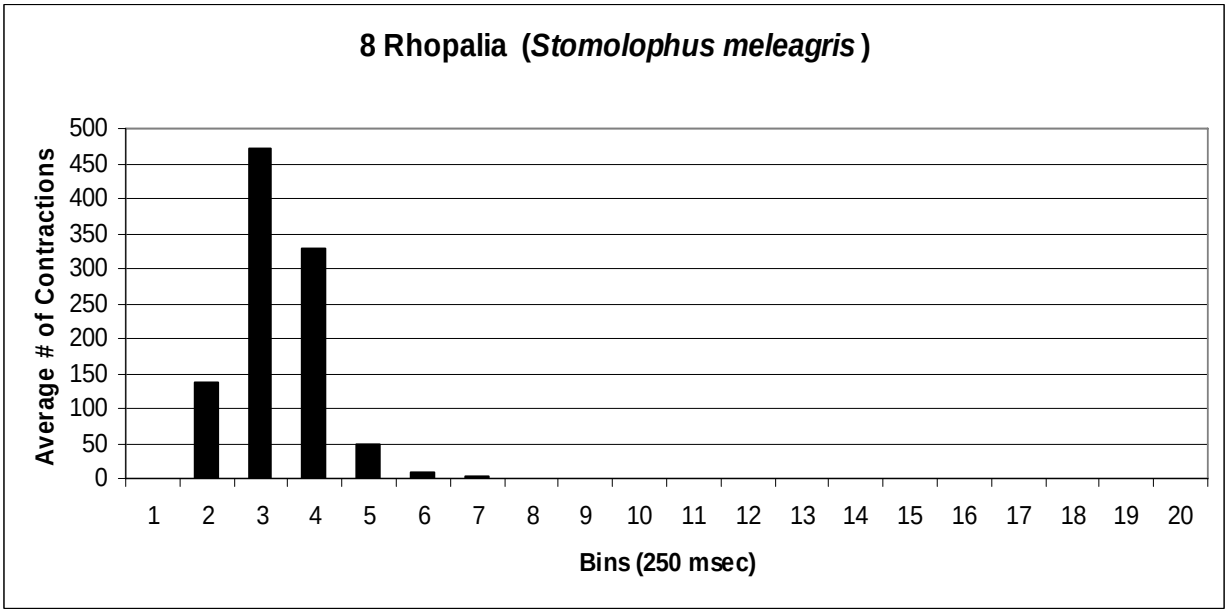


Figure 11. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for eight rhopalia trials (*Stomolophus meleagris*, n=16).

S.D. Despite this relationship, *Stomolophus* medusae were able to swim nearly as well with one rhopalium as with eight, due to their extreme overall excitability.

A comparison of real data to resetting, 50% resetting, and independent models constructed with *Stomolophus*-appropriate pacemakers showed a best fit with the resetting model, suggesting that the real pacemaker networks in *Stomolophus* are of the resetting type (fig. 16). There were significant differences when the cannonball data were compared to the independent and 50% resetting models ($P<0.001$); however there was not a significant difference when the data was compared to the 100% resetting model ($P>.05$; table 1).

Table 1. Comparison between the coefficient of variations of the models (independent- 0% resetting, semi independent -50% resetting, and 100% resetting) to the coefficient of variations of the animals.

Aurelia aurita (adult moon jellyfish)

Comparison	P value
Moon jellyfish vs. Independent	*** P<0.001
Moon jellyfish vs. 50% Resetting	ns P>0.05
Moon jellyfish vs. 100% Resetting	** P<0.01

Ephyra of *Aurelia aurita*

Comparison	P value
Ephyra vs. Independent	*** P<0.001
Ephyra vs. 50% Resetting	ns P>0.05
Ephyra vs. 100% Resetting	ns P>0.05

Chrysaora quinquecirrha (Sea Nettles)

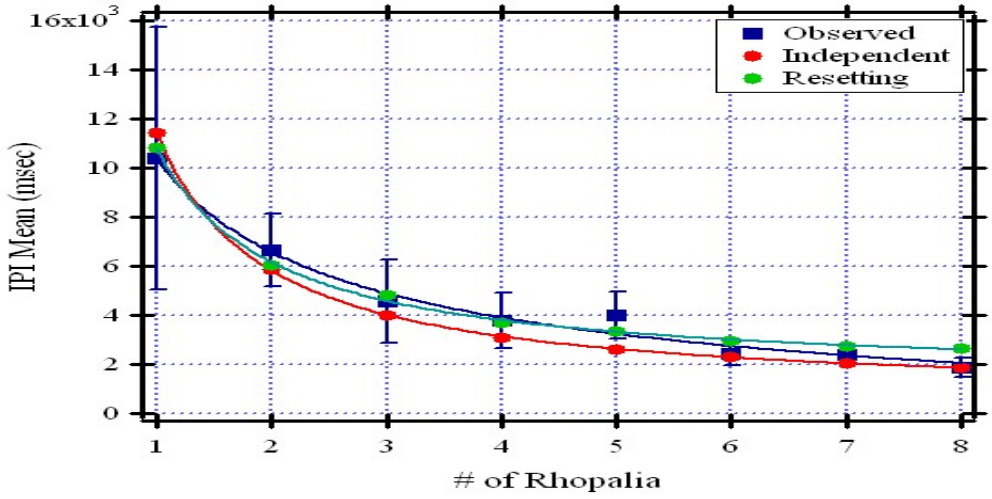
Comparison	P value
Sea Nettles vs. Independent	*** P<0.001
Sea Nettles vs. 50% Resetting	** P<0.01
Sea Nettles vs. 100% Resetting	ns P>0.05

Stomolophus meleagris (Cannonball)

Comparison	P value
Cannonball vs. Independent	*** P<0.001
Cannonball vs. 50% Resetting	*** P<0.001
Cannonball vs. 100% Resetting	ns P>0.05

*Unpaired t test with Welch correction; paired t test.
*** Extremely significant; ** very significant; ns – not significant

A



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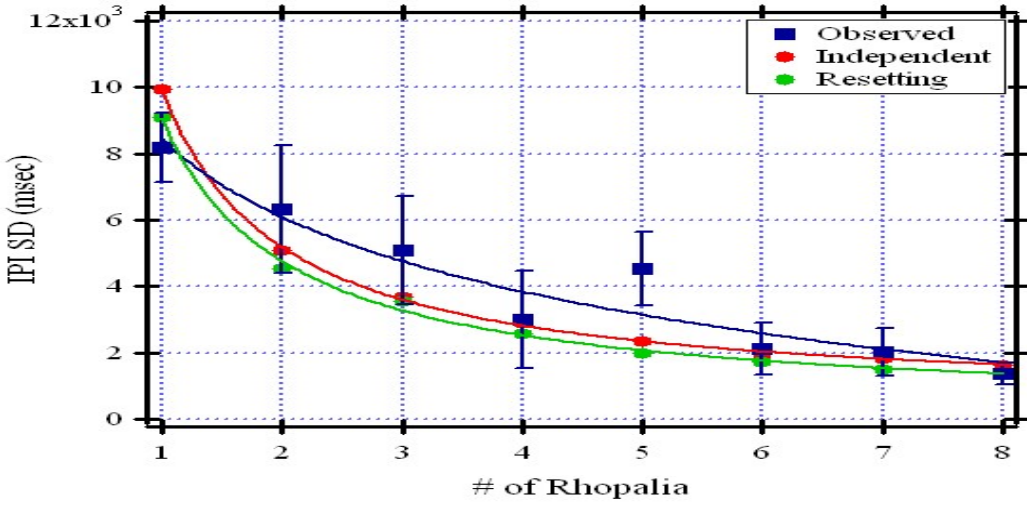

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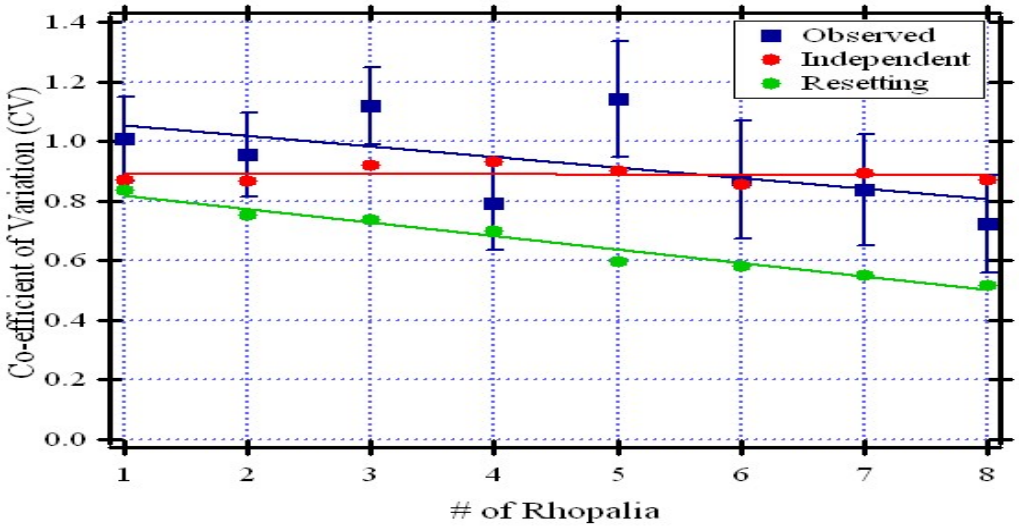


Figure 12. Relationship between the mean interpulse interval (IPI) (A), the standard deviation (S.D.) of the interpulse interval (B), and the coefficient of variation (CV) (C) and the number of pacemakers for the observed data from *Aurelia aurita*, a resetting pacemaker model and an independent pacemaker model.

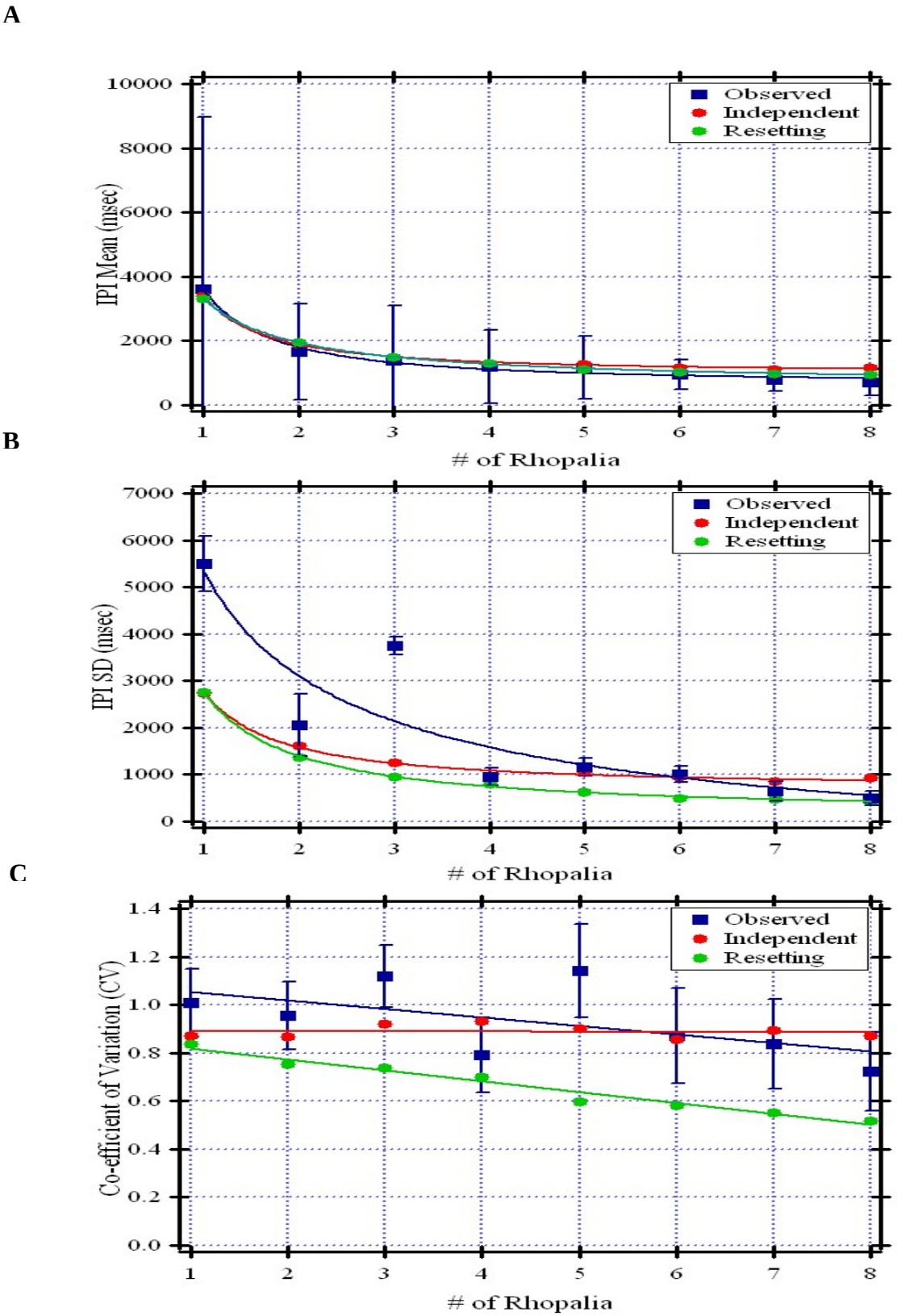


Figure 13. Relationship between the mean interpulse interval (IPI) (A), the standard deviation (S.D.) of the interpulse interval (B), and the coefficient of variation (CV) (C) and the number of pacemakers for the observed data from the ephyra of *Aurelia aurita*, a resetting pacemaker model and an independent pacemaker model.

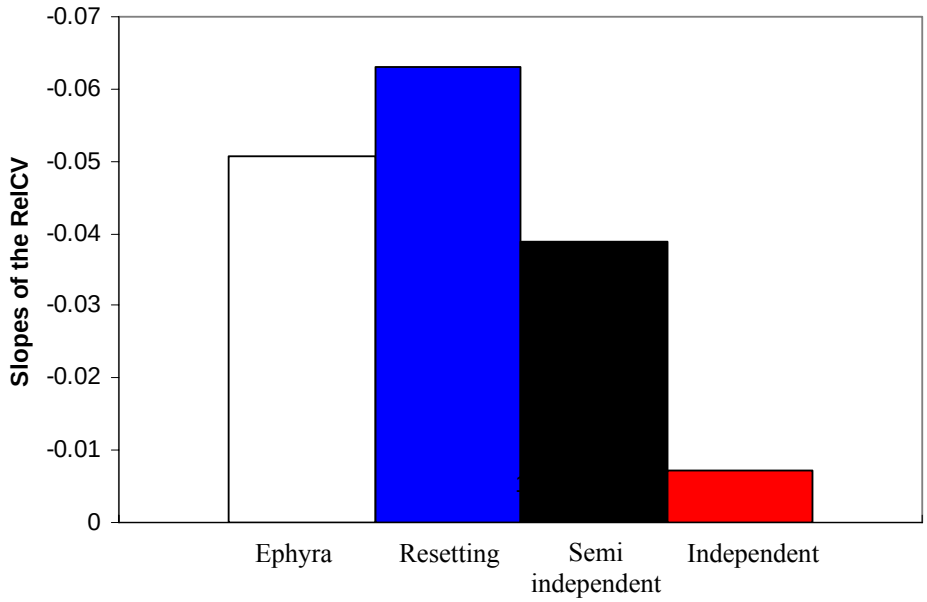


Figure 14. Slopes of the coefficient of variation for the ephyra of Aurelia aurita against the different models (resetting, semi independent, and independent models).

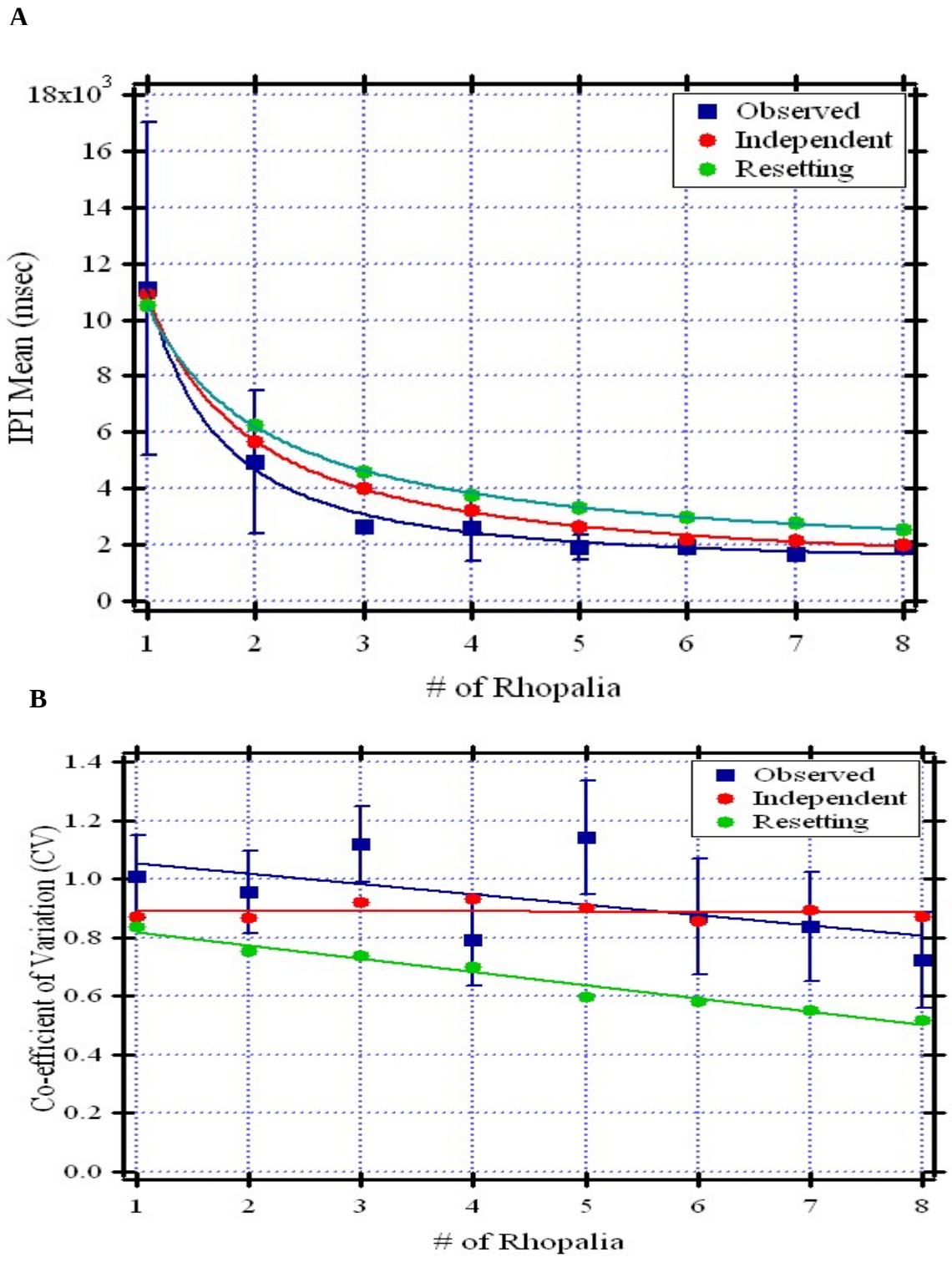


Figure 15. Relationship between the mean interpulse interval (IPI) (A), and the coefficient of variation (CV) (B), and the number of pacemakers for the observed data from *Chrysaora quinquecirrha* (B), a resetting pacemaker model and an independent pacemaker model.

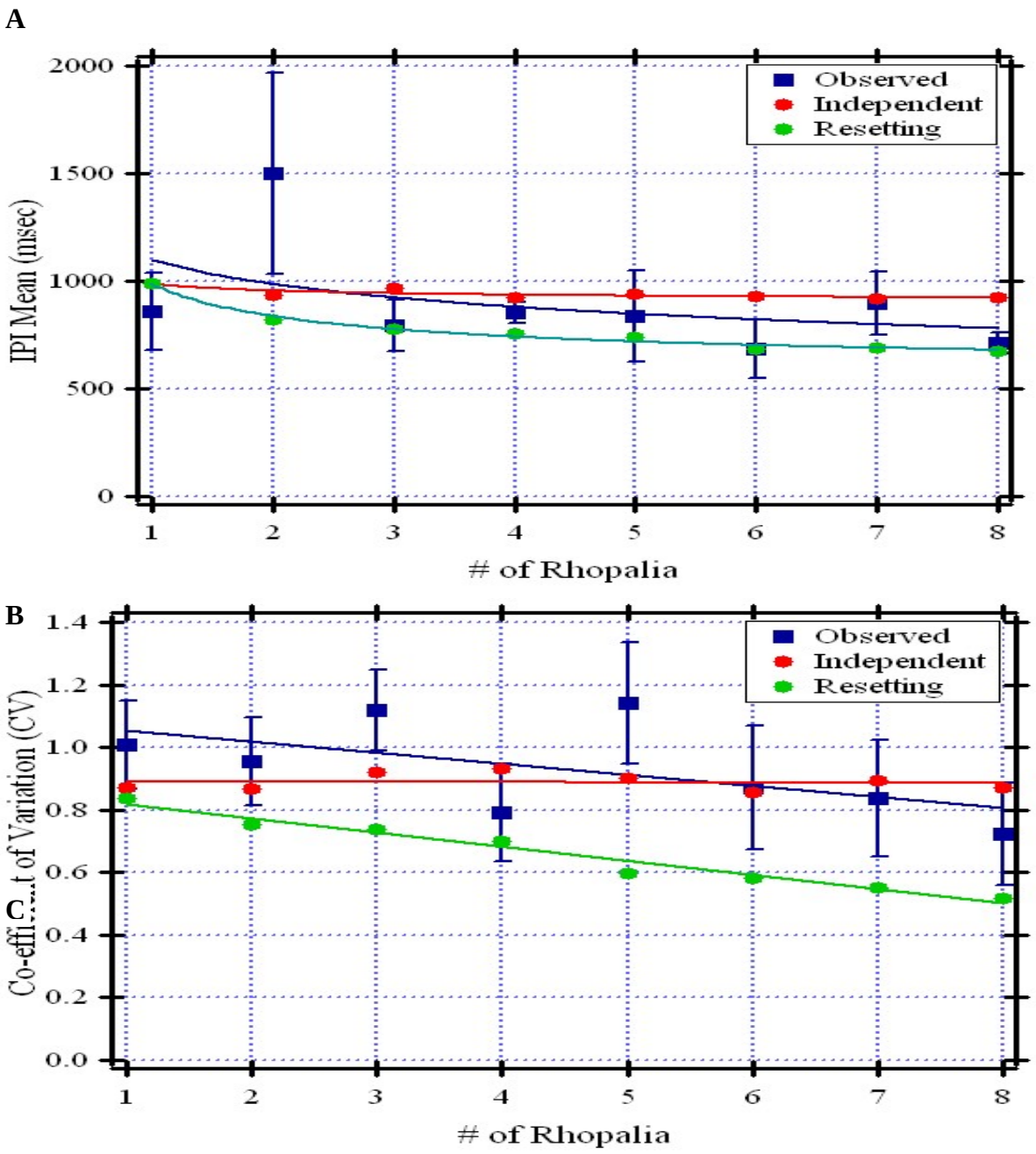


Figure 16. Relationship between the mean interpulse interval (IPI) (A), and the coefficient of variation (CV) (B), and the number of pacemakers for the observed data from *Stomolophus meleagris*, a resetting pacemaker model and an independent pacemaker model.

DISCUSSION

The experimental data from ablation studies presented here confirm that when only a single rhopalium produce irregular patterns of pacemaker impulses to the swim systems, which are characterized by large means (compared to the intact animals) and large standard deviations. However, the records from one-rhopalium preparations also indicate that they can produce very regular pacemaker output for variable periods of time. In particular, a single rhopalium can control the intact swim system if it receives enough excitatory sensory or modulatory input (Lerner et al., 1971). Horridge (1959) showed that when an individual rhopalium in *Aurelia aurita* was artificially stimulated, the jellyfish produced rapid and regular swim contractions. Horridge (1959) also showed that an individual pacemaker (rhopalium) had an average frequency that was less than half its maximal frequency.

Activities of individual pacemakers, as well as multiple pacemakers, were tested by using ablation experiments. Similar to the observations of Lerner et al., (1971), the redundancy of pacemakers permitted swimming to continue despite the removal of several pacemakers. The results presented here are in agreement with those of Horridge (1954b), Lerner et al., (1971), and Murray (1977), which showed that multiple pacemakers increase both the overall swim frequency and the regularity of swimming.

Maintaining a regular swim output is important for efficient neuromuscular activity since the swim muscles of scyphozoan jellyfish have been shown to produce tension that depended heavily on frequency-dependent facilitation (Bullock, 1943). If the swim frequency is too low, facilitation is reduced or non-existent, producing weaker contractions. Whereas higher frequencies, contractility increases. Similar results were obtained for other jellyfish, with the efficiency of the swim musculature tied to similar frequency-dependent facilitatory mechanisms

(Satterlie and Nolen, 2001). In particular, the strength of contractions during normal swimming in intact cubomedusae was found to be around 80% of the maximal contraction strength (Satterlie, 1979).

The question addressed here is how multiple pacemakers are linked to control a common effector sheet (swim musculature) in scyphozoan jellyfish. Early observations gave rise to the concept of a resetting network, and early models used this assumption to further demonstrate the value of multiple pacemakers in the scyphozoan swim system (Horridge, 1959; Lerner et al., 1971; Murray, 1977). However, two observations underline the need to further examine pacemaker linkages in these jellyfish. First, an artificial network in which pacemakers have absolutely no interactions (independent network) will still produce an increase in regularity and overall frequency when pacemakers are added to the system, even though the overall variability will be less than that in a resetting network (Satterlie and Nolen, 2001). Second, a similar modeling analysis of pacemaker interactions in cubomedusan jellyfish demonstrated that the pacemakers are linked in a semi-independent network, in which the connections are resetting, but individual pacemakers are sometimes freed from the resetting influence (they operated independently; Satterlie and Nolen, 2001).

Aurelia aurita

Instead of the *Aurelia aurita* having a resetting pacemaker network like previously thought (Lerner et al., 1971; Murray, 1977), the model shows that they have a semi-independent network similar to cubomedusae. The advantage of having a semi-independent network is that it reduces the variability in swim frequency, and at the same time, increases the flexibility of swimming (Satterlie and Nolen, 2001). Having a semi-independent network also allows *Aurelia*

aurita to benefit from both the resetting and independent pacemaker networks. Compared to the other species of jellyfish, *Aurelia* exhibits the highest degree of flexibility while *Stomolophus* exhibits the least degree of flexibility, which further supports the semi-independent network in the former.

The method of feeding may also play a critical role in the organization of the pacemaker network as *Aurelia* uses its short marginal tentacles to feed and its oral arms are mainly used to move prey into its gastrovascular cavity. This type of feeding may require more flexible swimming behavior since the marginal tentacles are not set out to "fish" for prey at a great distance from the bell margin.

Aurelia aurita ephyra

The pacemaker network of the ephyra of *Aurelia aurita* differs from that of the adults in that it is of the resetting type. The morphology of the ephyra changes dramatically as it makes the transition into its adult form, as does swimming behavior. In particular, the ephyra are negatively buoyant, so swimming regularity is advantageous to staying in the water column. The ephyra do have a higher swim frequency, and this has been suggested to be a requirement for remaining in the water column in order to feed (Horridge, 1956). The ephyra lack marginal tentacles, and must relay on their manubrium to catch prey.

Chrysaora quinquecirrha

Chrysaora quinquecirrha has a resetting pacemaker network. They are voracious predators that use long marginal tentacles as well as extremely long oral arms to collect prey. Both tentacles and oral arms can extend a meter from the swimming bell. This allows more of a

passive prey apprehension mechanism, although swimming currents still help in directing prey to the feeding structures (Matanoski and Hood, 2006). Because of the length of the tentacles and oral arms, changing in swimming activity (as from a more flexible swimming system) will not be directly or quickly transferred to the tentacles.

Stomolophus meleagris

As mentioned above, having pacemakers that interact through resetting linkages is advantageous if swim regularity and high swim frequency is favored over swim flexibility (Satterlie and Nolen, 2001). *Stomolophus meleagris* display 100% resetting behavior, which may be a consequence of the rigidity of the swimming bell of Only the lower portions of the bell contract during swimming activity, so less surface area is available for large asymmetric contractions, which are necessary for turning behavior. In addition, a high swim frequency is necessary to prevent *Stomolophus* from sinking. Its feeding behavior involves use of rather elaborate oral arms since *Stomolophus* lacks marginal tentacles. Water circulation through the oral arms and swimming bell enhances feeding, rather than directional movement of the medusa through the water. In this instance, a resetting pacemaker network would better serve both swimming and feeding behavior.

In contrast, in an independent pacemaker network that has many pacemakers (in this case scyphomedusae) would produce high variability and shorter interpulse intervals (Satterlie and Nolen, 2001). Jellyfish that have an independent pacemaker network can potentially have inefficient locomotion due to high variability of swim contractions (Satterlie and Nolen, 2001). The coefficient of variation (CV) would also have to increase as pacemakers are added to the system (Satterlie and Nolen, 2001). In the three species of the scyphomedusae, the coefficient of

variation did not increase as pacemakers were added to the system therefore, we can rule out an independent network in these scyphozoan species.

Both resetting and semi independent pacemaker networks are found in scyphomedusae, and the type of network may be related to the ecology of the species. This is interesting since the basic organization of the rhopalia pacemakers is consistent within the class Scyphozoa, while their connectivity may be species specific, and even developmentally specific.

LITERATURE CITED

Anderson, P. and Schwab, W. 1982. Recent advances and model system in coelenterate neurobiology. *Progress in neurobiology*. 19:213-236.

Anderson, P. and Schwab, W. 1983. Action potentials in neurons of motor nerve net of *Cyanea* (Coelenterata*). Journal of Neurophysiology*. 50:671-683.

Anderson, P. and Schwab, W. 1984. An epithelial cell-free preparation of the motor nerve net of *Cyanea* (Coelenterata: Scyphozoa). *Biological Bulletin*. 166:396-408.

Bullock, T. 1943. Neuromuscular facilitation in scyphomedusae. *J. Cell Comp. Physiol*. 22:251-272.

Bullock, T. and Horridge, G. 1965. *Structure and function in the nervous systems of invertebrates.* Vol. 1. San Francisco: W.H. Freeman and Co.

Carlberg, M., Alfredsson, K., Nielsen, S., and Anderson, P. 1995. Taurine-like immunoreactivity in the motor nerve net of the jellyfish *Cyanea capillata*. Biological Bulletin. 188:78-82.

Chapman, D. 1999. Microanatomy of the bell rim of *Aurelia Aurita* (Cnidaria: Scyphozoa). *Can. J. Zoology*. **77**:34-46.

Dayan, P. and Abbott, L. 2001. *Theoretical Neuroscience: Computational and Mathematical Modeling of Neural Systems.* Cambridge, Massachusetts: The MIT Press.

Eiane, K., Aksnes, D., Bagoien, E., and Kaartvedt, S. 1999. Fish or Jellies-A Question of Visibility? *Limnology and Oceanography*. 44:1352-1357.

Ford, M., Costello, J., Heidelberg, K., and Purcell, J. 1997. Swimming and feeding by the scyphomedusa *Chrysaora quinquecirrha. Marine Biology*. 129:355-362.

Gladfelter, W. 1972. Structure and function of the locomotory system of the Scyphomedusa *Cyanea capillata*. *Marine Biology*. 14:150-160.

Horridge, G. 1954a**.** Observations on nerve fibers of Aurellia aurita. *Q.J. Microsc . Sci*. 95:85-92.

Horridge, G. 1954b**.** The nerves and muscles of medusae. I. Conduction in the nervous system of *Aurellia Aurita* Lamarck. *Journal of Experiment Biology*. 31:594-600.

Horridge, G. 1956. The nerves and muscles of medusae. V. Double innervation in Scyphozoa. *The Journal of Experimental Biology*. 33:366-383.

Horridge, G. 1959. The nerves and muscles of medusae. VI. The rhythm. *The Journal of Experimental Biology*. 36:72-91.

Lerner, J., Mellen, S. A., Waldron, I., and Factor, R.M. 1971. Neuronal redundancy and regularity of swimming beats of scyphozoan medusae. *The Journal of Experimental Biology*. 55:177-184.

Matanoski, J. and Hood, R. 2006. An individual-based numerical model of medusa swimming behavior. *Marine Biology*. 149:595-608.

Matanoski, J., Hood, R., Purcell, J. 2001. Characterizing the effect of prey on swimming and feeding efficiency of scyphomedusa *Chrysaora quinquecirrha*. *Marine Biology*, 139:191-200.

Murray, M. 1977. Systems of Mutally-triggering Events Generators: Basic Properties and Functions in Information Transmission and Rhythm Generation. *Journal of Comparative Biology*. 177:63-98.

Pantin, C., and Dias, M. 1952. Rhythm and afterdischarge in medusae. *Acad. Brazil Cien*. 24:351-364.

Passano, L. 1965. Pacemakers and activity patterns in medusae: Homage to Romanes. *American Zoologist*. 5:465-481.

Passano, K. and Passano, L. 1970. The endodermal nerve net of scyphozoa. *J. morph.* 133:105-124.

Romanes, G. 1876. Preliminary observations on the locomotor system of Medusa. *Phil Trans Roy Soc. London*. 166:269-313.

Romanes, G. 1877. Further observations on the locomotor system of medusae. *Phil. Trans. R. Soc*. 167:659-752.

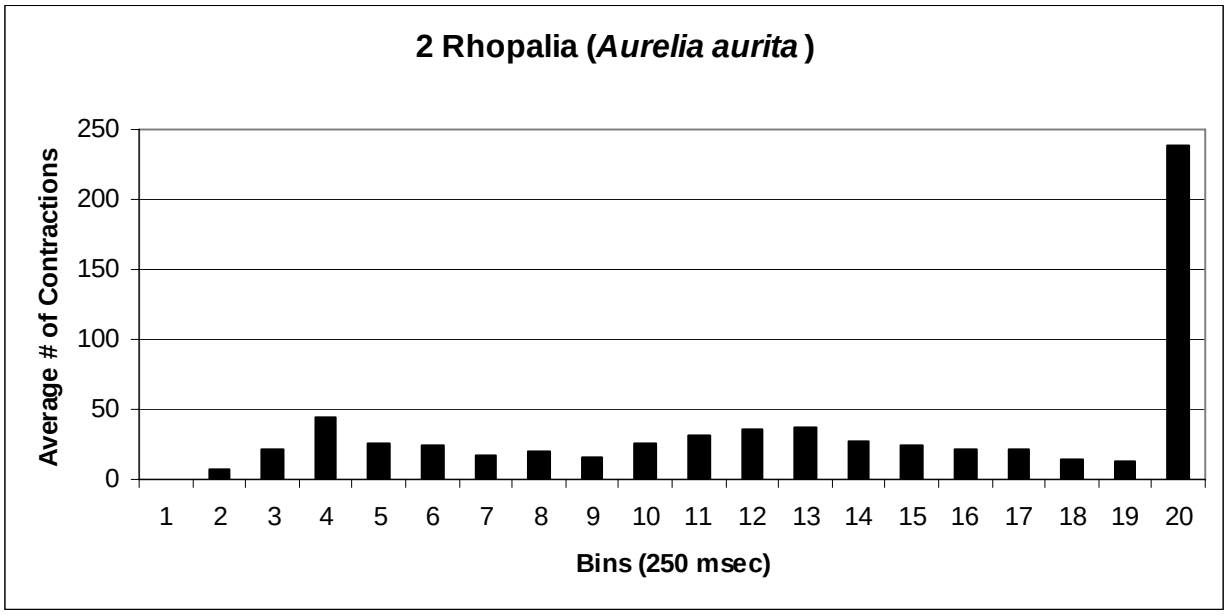
Satterlie, R. and Nolen T. 2001. Why Do Cubomedusa have only Four Swimming Pacemakers? *The Journal of Experimental Biology*. 204:1413-1419.

Satterlie, R. 2002. Neuronal control of swimming in jellyfish: a comparative story. *Can. Journal of Zoology*. 80:1654-1669.

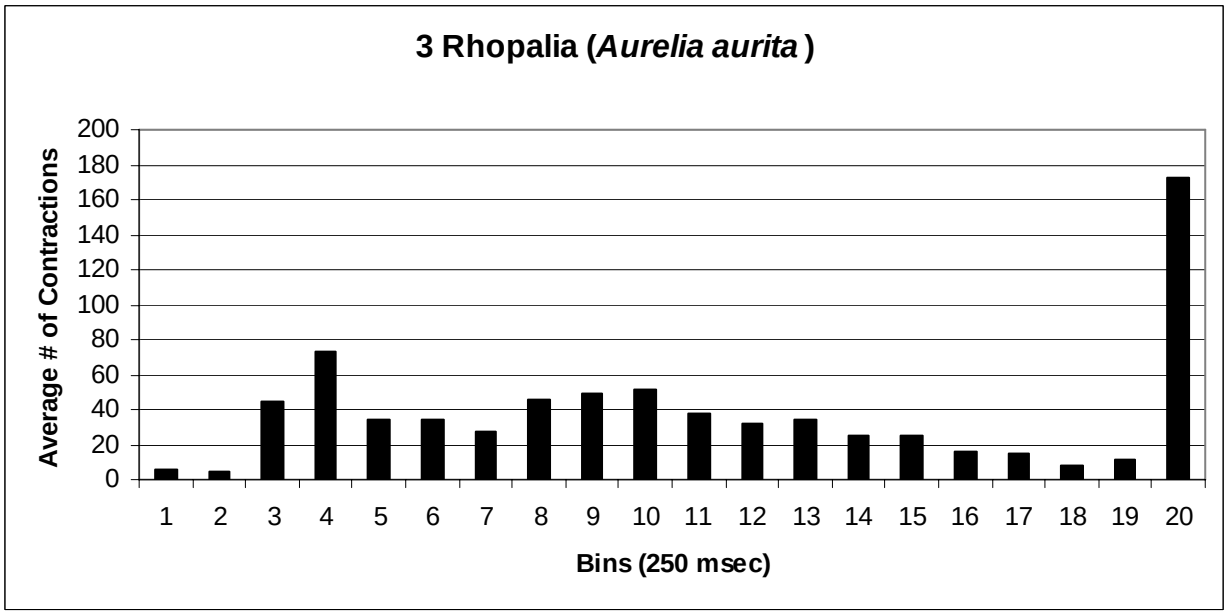
Satterlie, R. and Spencer, A. 1979. Swimming control in cubomedusan jellyfish. *Nature*. 281:141-142.

Satterlie, R. and Spencer, A. 1987. *Organization of conducting systems in "simple" invertebrates: Porifera, Cnidaria, and Ctenophora. Nervous Systems in Invertebrates*. M.A. Ali. Plenum 213-264.

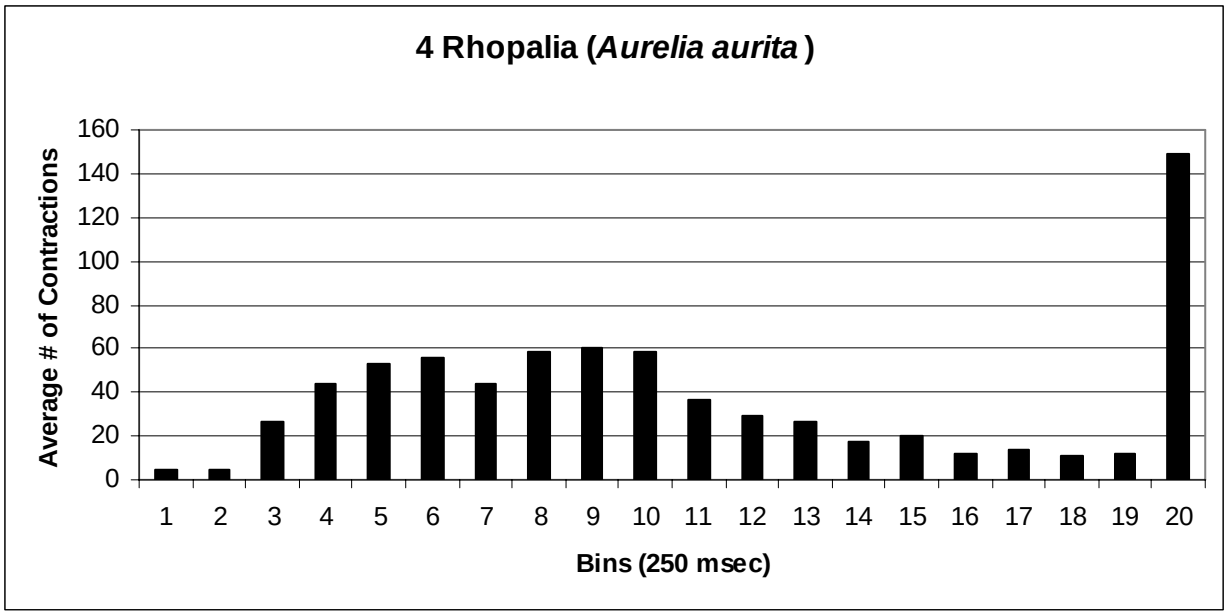
APPENDIX



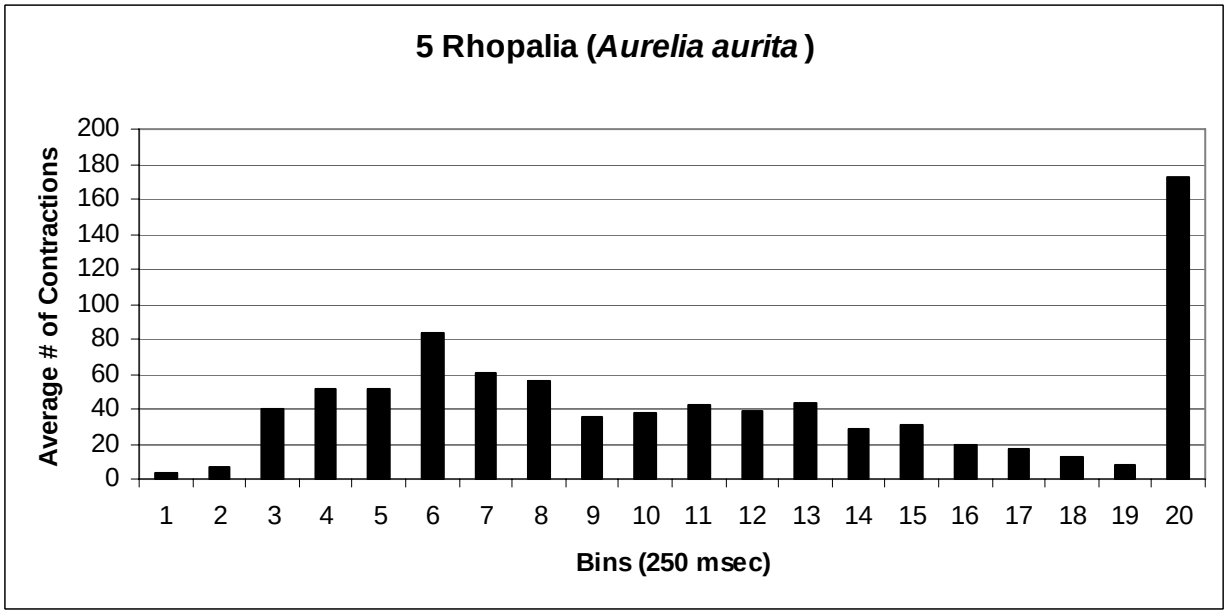
A. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for two rhopalia trials (*Aurelia aurita*, n=6). Note that bin 20 = >4,750 msec.



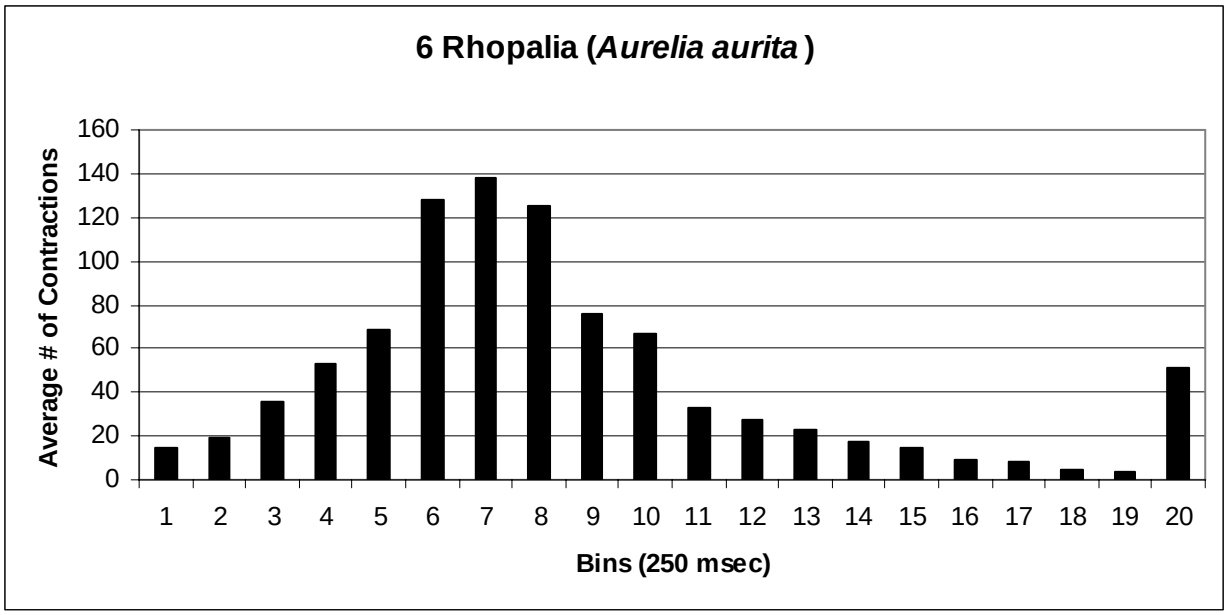
B. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for three rhopalia trials (*Aurelia aurita*, n=6).



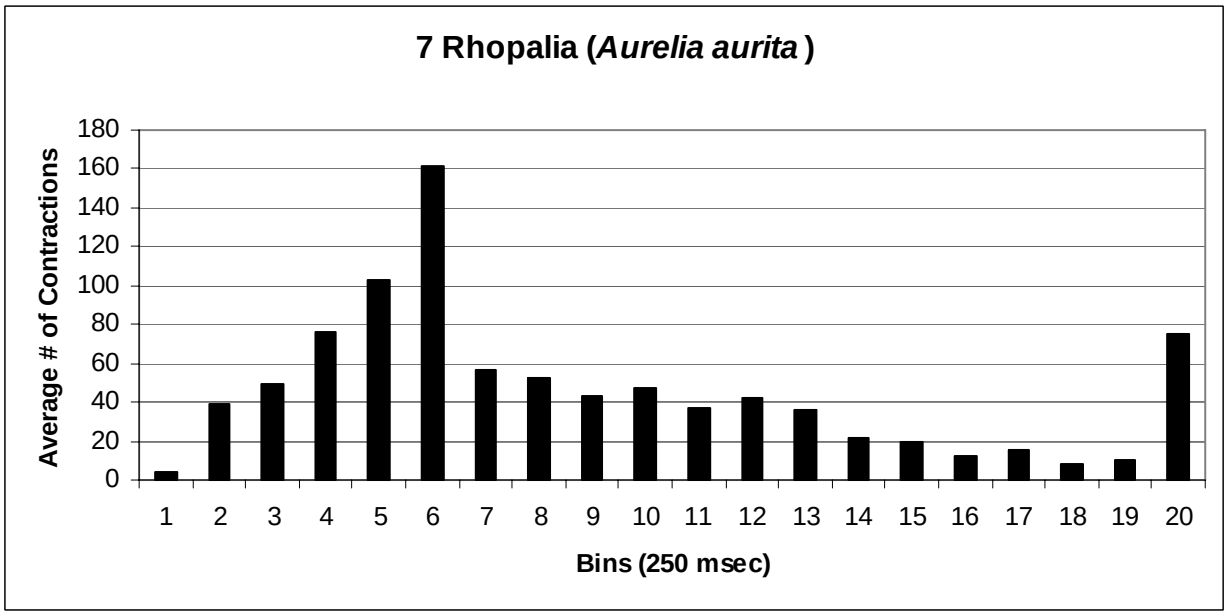
C. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for four rhopalia trials (*Aurelia aurita*, n=6). Note that bin 20 = >4,750 msec.



D. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for five rhopalia trials (*Aurelia aurita*, n=7).



E. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for six rhopalia trials (*Aurelia aurita*, n=8). Note that bin 20 = >4,750 msec.



F. Interpulse interval (IPI) data from experimental animals. The interpulse intervals were separated into 250 msec bins. Mean number of contractions for seven rhopalia trials (*Aurelia aurita*, n=10).