Slides and Oral Remarks Presented in:

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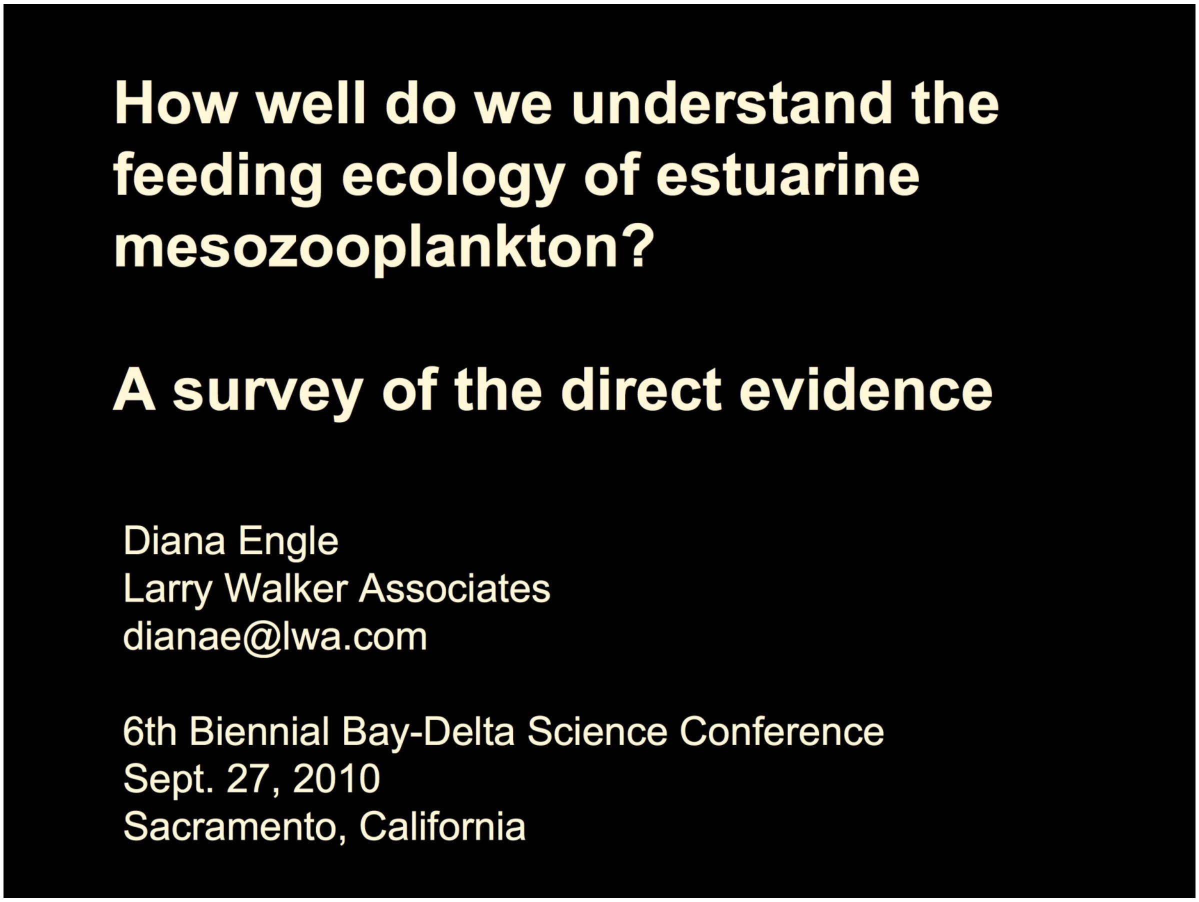
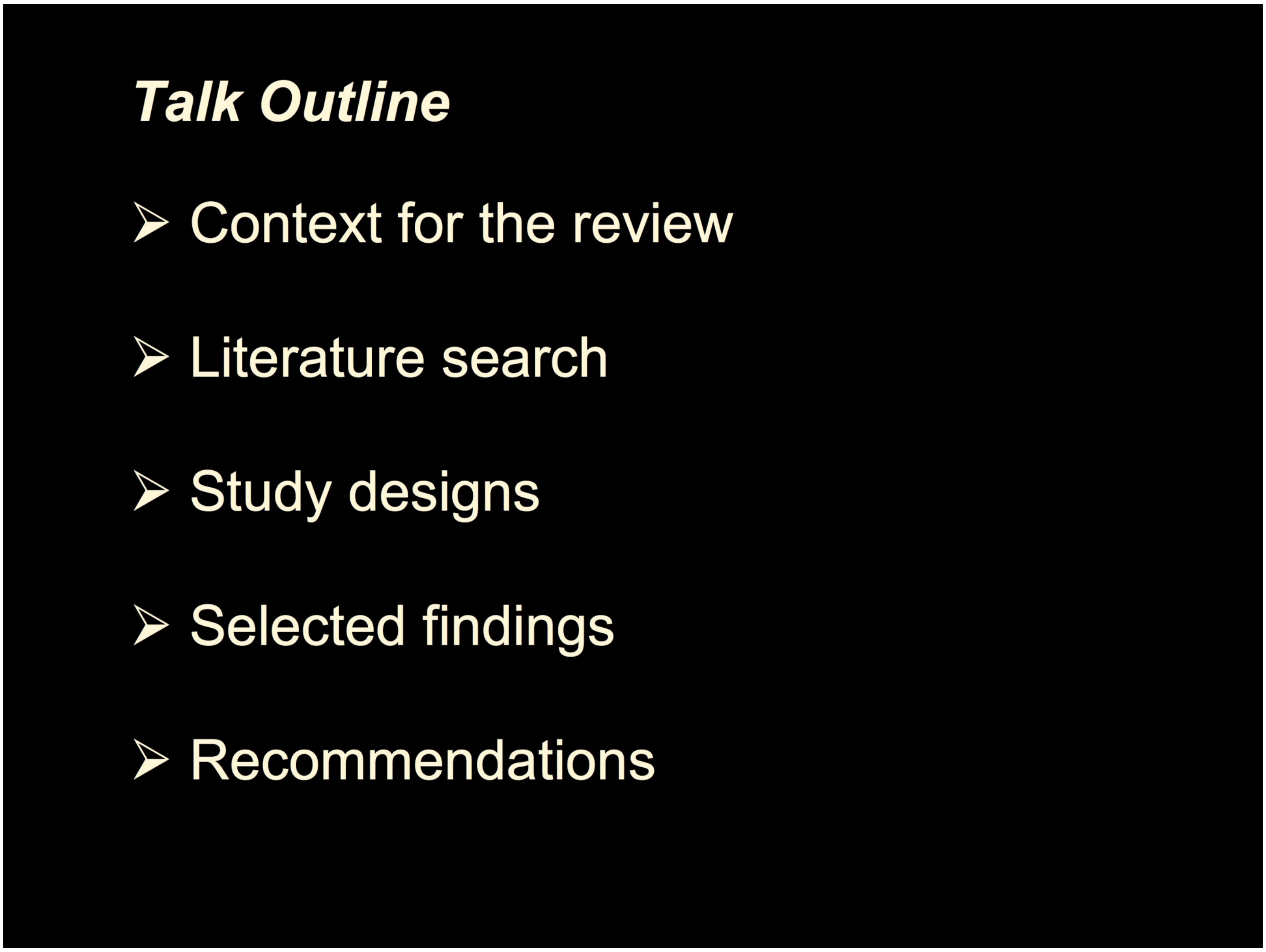
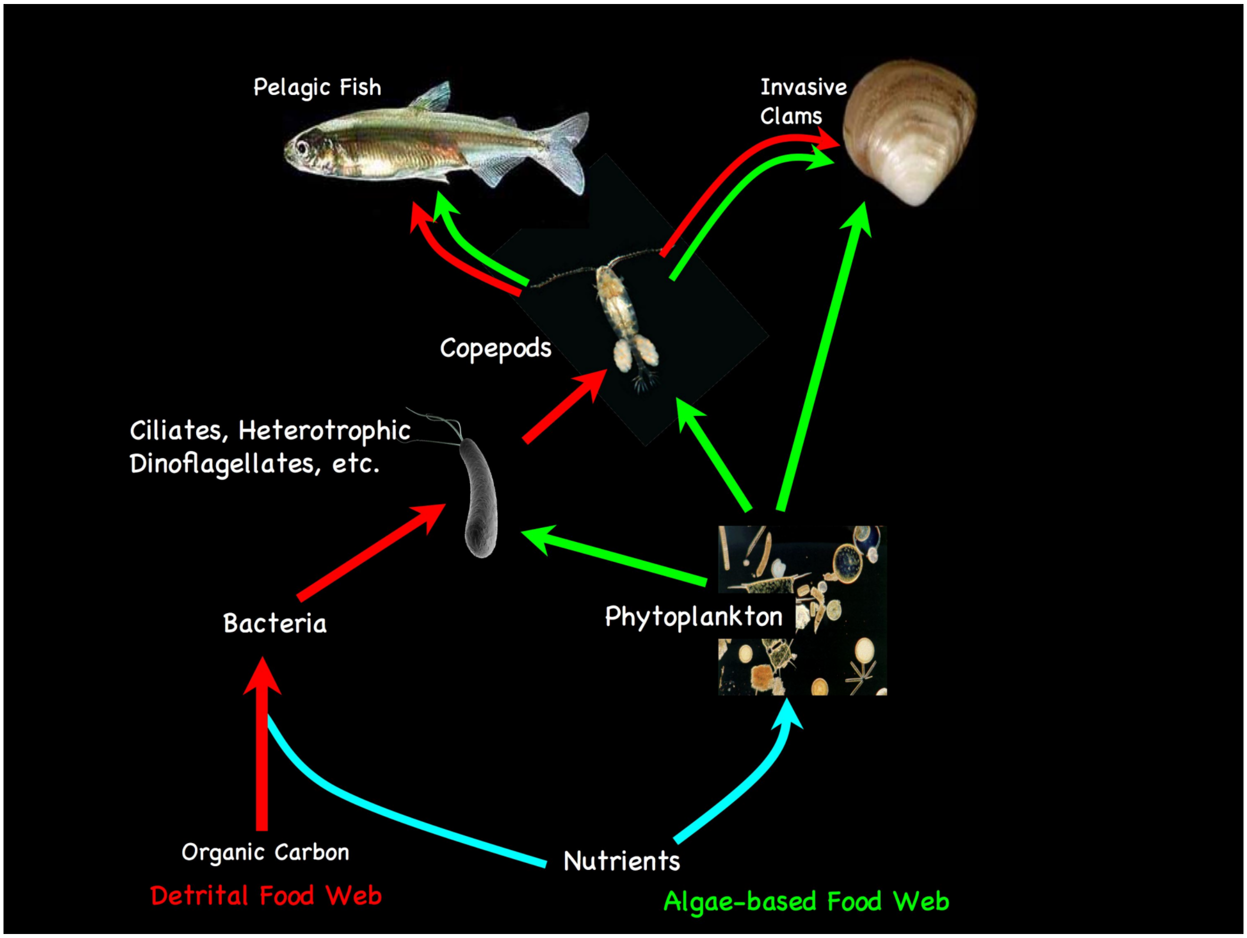


Today I'm going to share with you some findings from a review I conducted of the academic literature regarding direct feeding experiments that have been conducted using copepod species that are pertinent to the San Francisco Estuary.

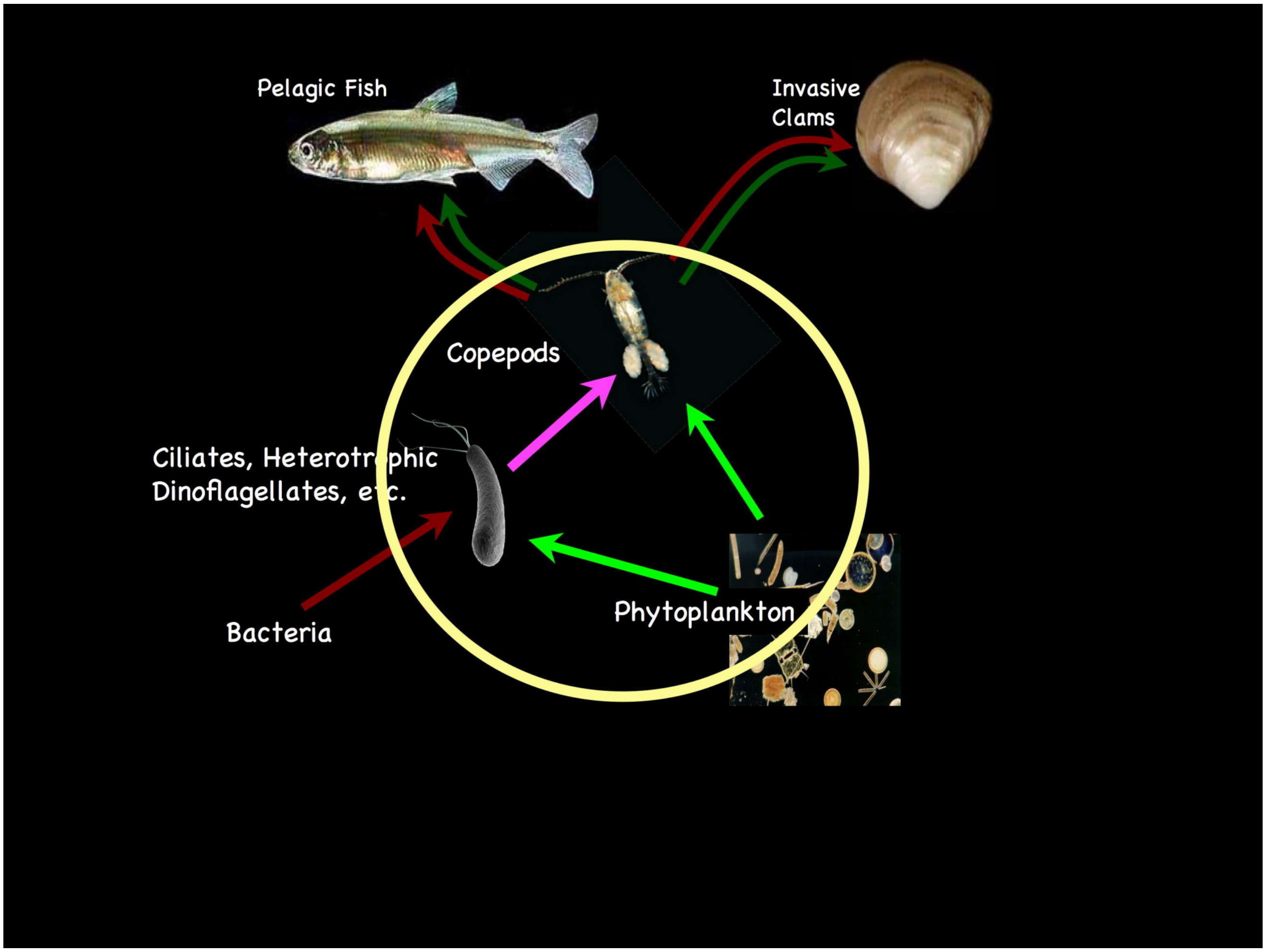


In my talk I will:

First provide a context for the review
Describe the Literature Search
Summarize the study designs
Share some selected findings
Offer some recommendations for future research

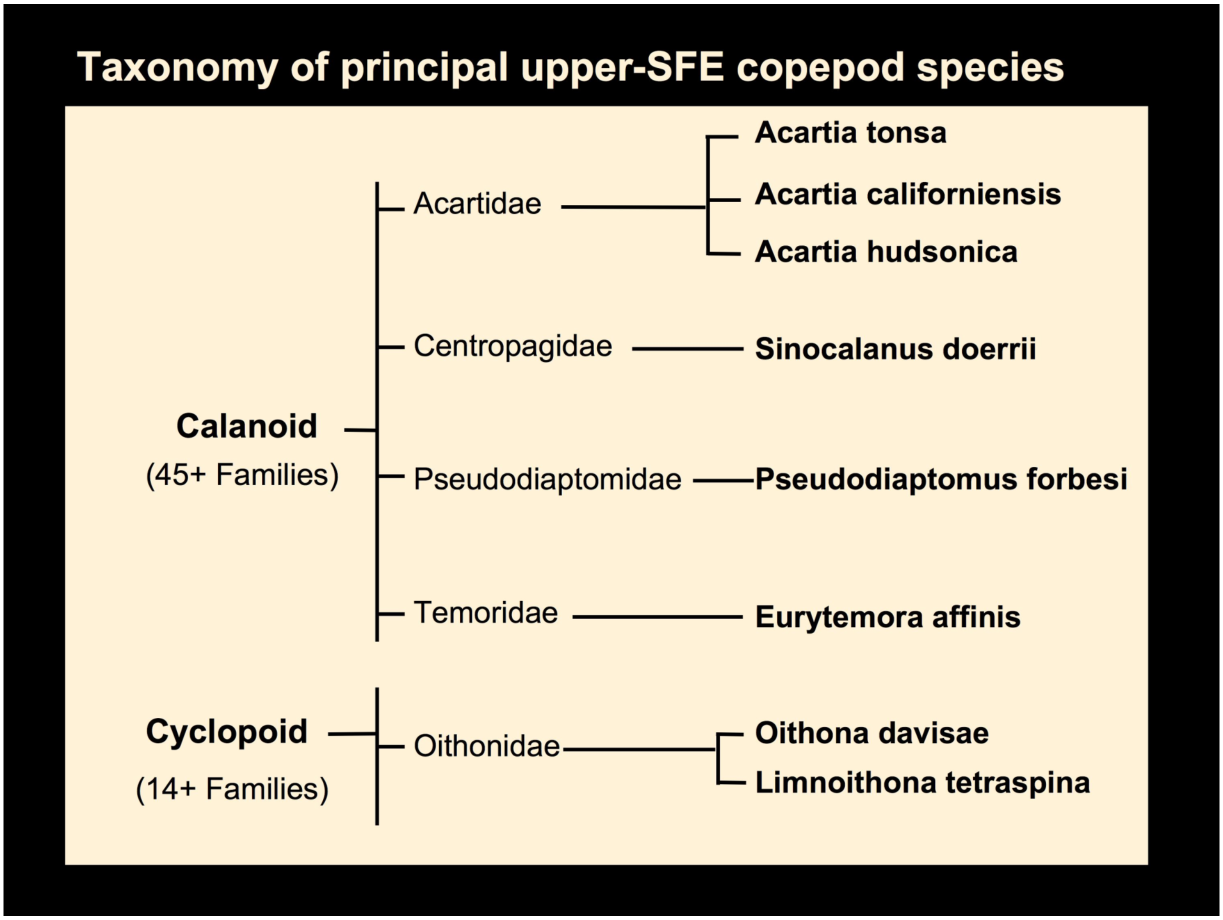


This is a simple respresentation of the pelagic food web in the upper SFE, showing how copepods belong to both the detrital food web and the algae-based food web, and reminding us that energy from both sources passes through copepods on its way to higher trophic levels such as pelagic fish. Although several of the copepods in the estuary are capable of feeding directly on phytoplankton - they have also been shown in several studies to prefer ciliates and other heterotrophic prey and also to select motile prey over non-motile prey.

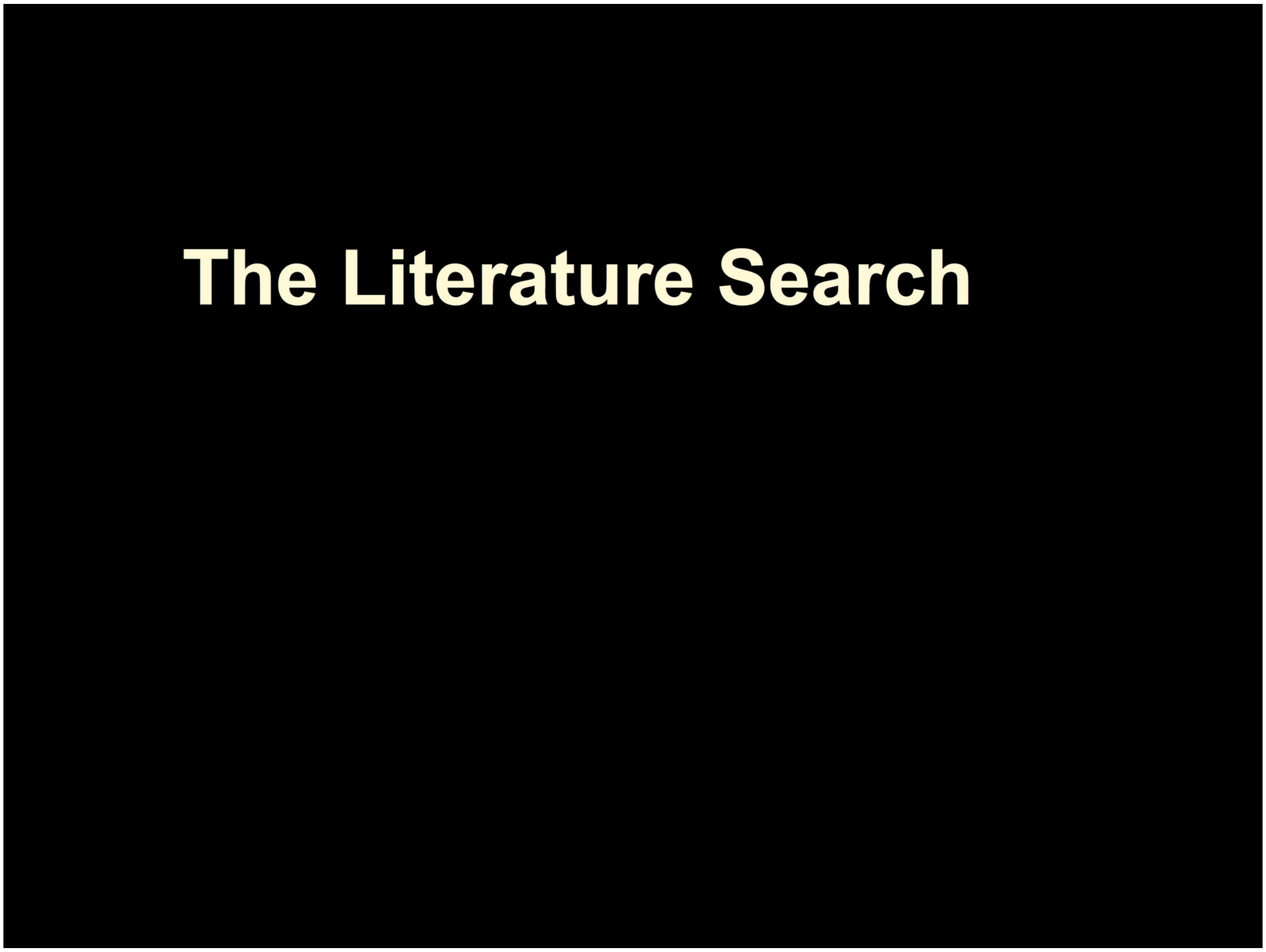


There is a common assumption that recent changes in phytoplankton composition in the estuary signal a deterioration in the amount or quality of food available to copepods, and ultimately to pelagic fish. For example, we are accustomed to hearing that large diatoms are good food for copepods, but small greens and other flagellated autotrophs are not. But there is evidence from several estuaries and marine systems that much of phytoplankton production - even during blooms - is funneled through small heterotrophs and then to copepods - in which case, does it matter as much which phytoplankton taxa occupy the prey spectrum?

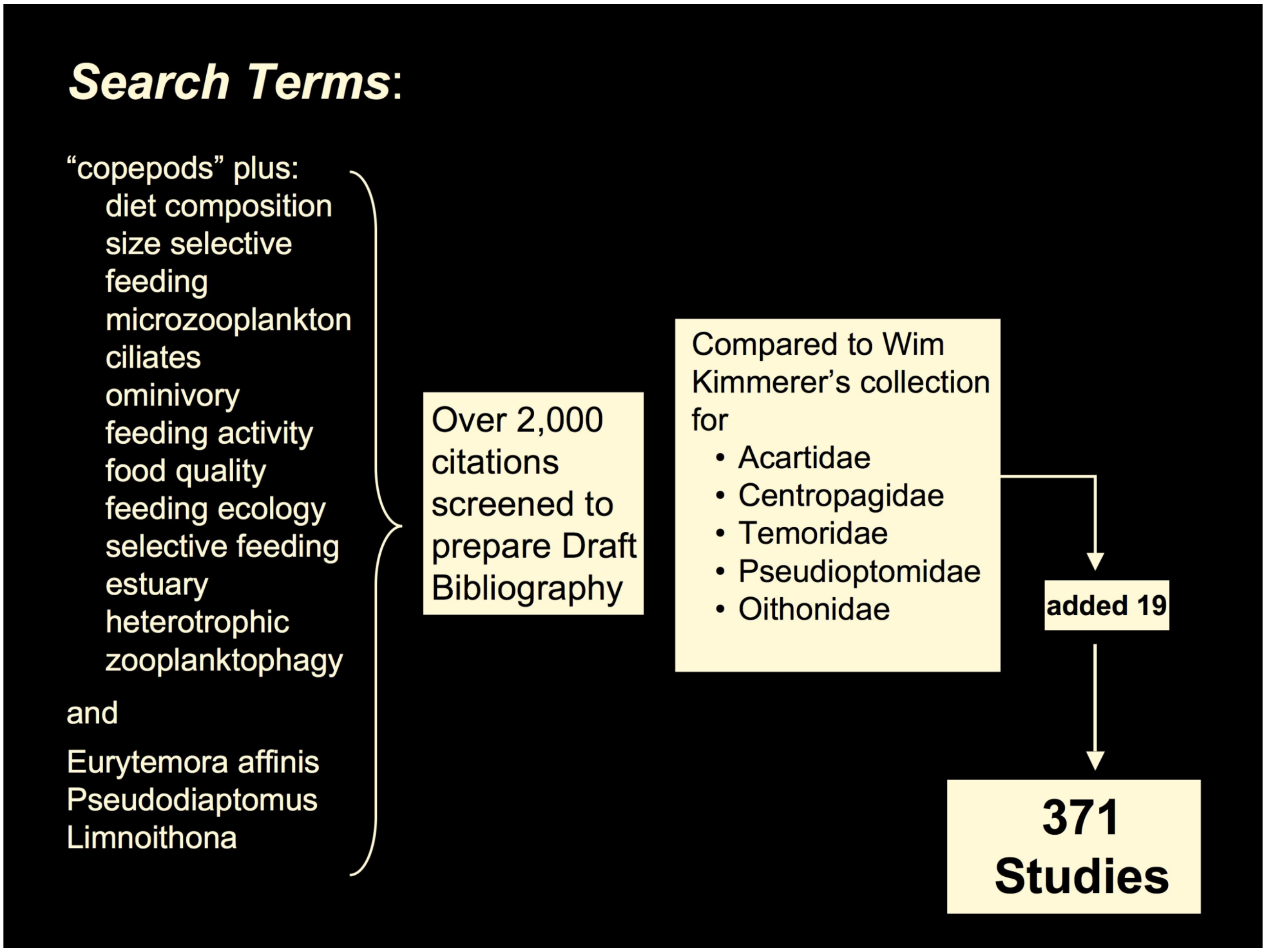
It was in this context I decided to survey the academic literature to see how well we are investigating the relative quality of the foods available to copepods in the estuary.



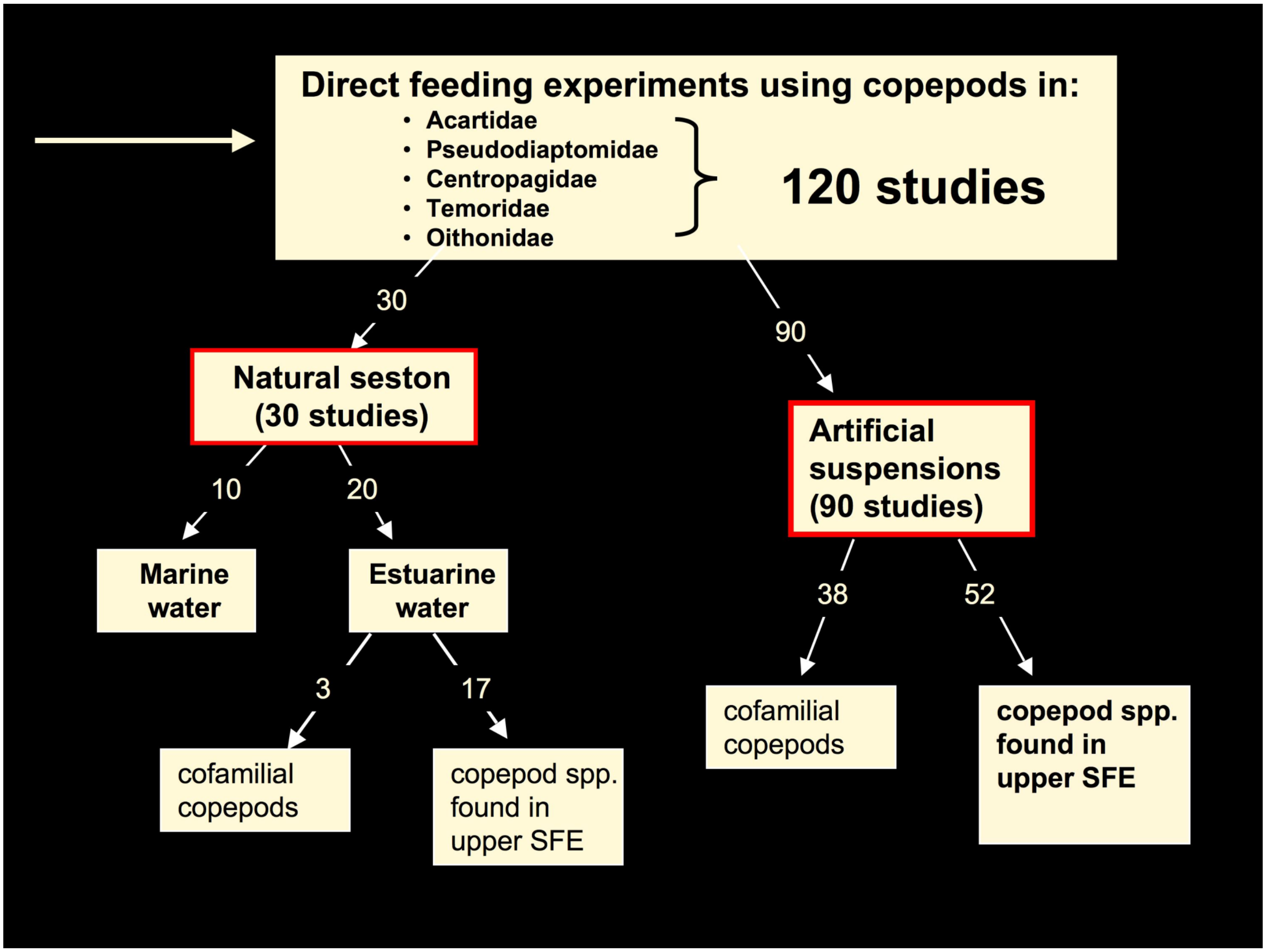
The right side of this diagram shows the 8 copepod species that are encountered with any regularity in the upper estuary. The 5 families they belong to are listed in the middle. Later in the talk, I will refer to copepods that are *cofamilial* with the upper SFE species. By that I mean species which don't occur in the upper estuary, but which belong to the five families in the slide.



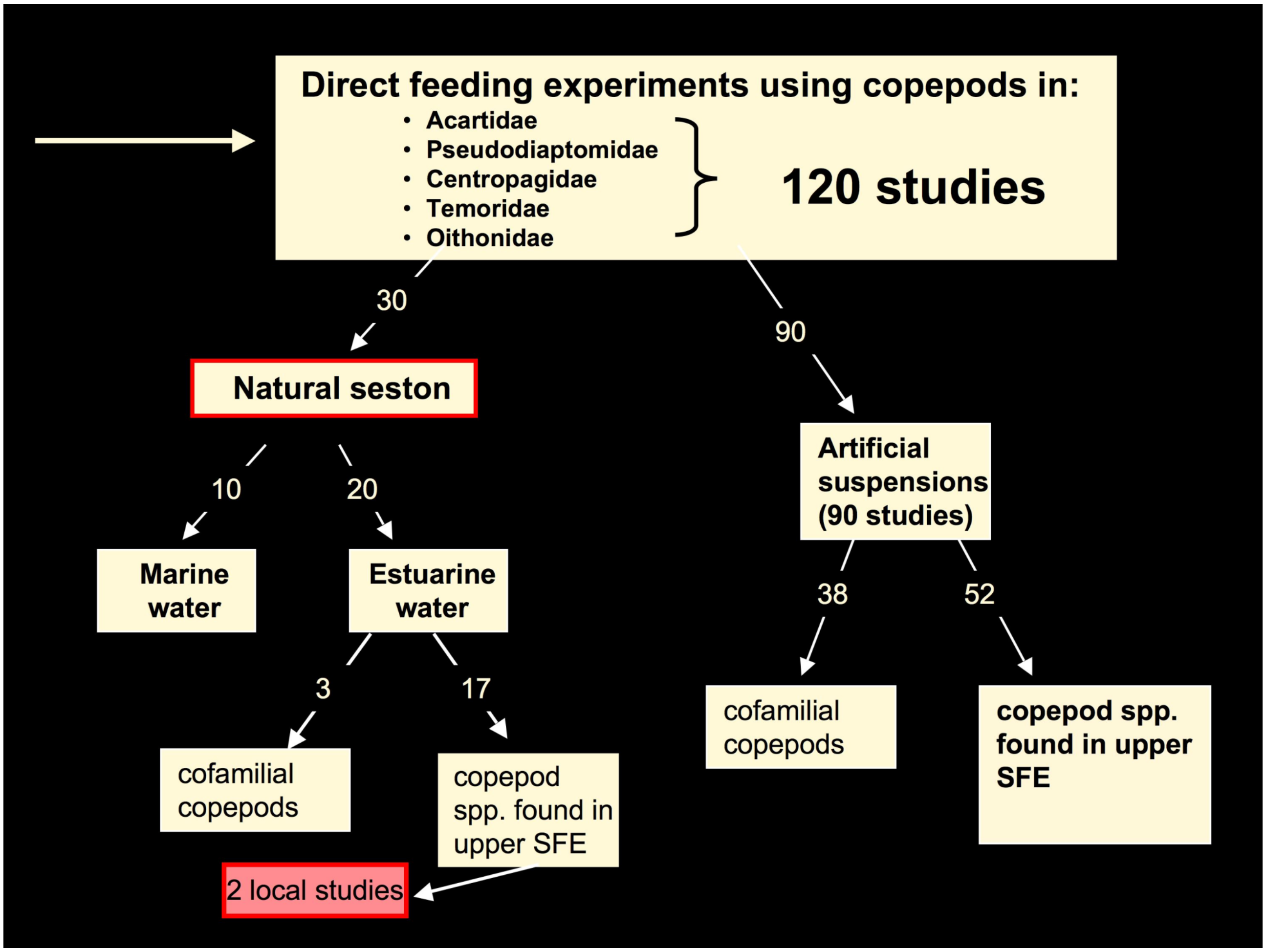
Now I'll describe the literature search



On the left side of the slide I list the search terms which were combined in a variety of ways to query the Web of Science. Over 2000 citations resulting from these searches were screened to produce a Draft Bibliograpy. This was compared to Wim Kimmerer's collection of articles retrieved by using the 5 pertinent copepod families as search terms in his Endnote library. This resulted in addition of 19 articles I had missed in my own searches, for a total of 371 studies.



I next evaluated the methods that were used in the 371 studies in my library. 120 studies turned out to be direct feeding tests using copepods in the five pertinent families. These can be further categorized as shown in the diagram. You can see that there are 3 time more experiments using artificial suspensions than natural seston for these 5 copepod families.



2 natural seston feeding studies from the SFE were published in the last few years

Local Investigations w. Natural Seston

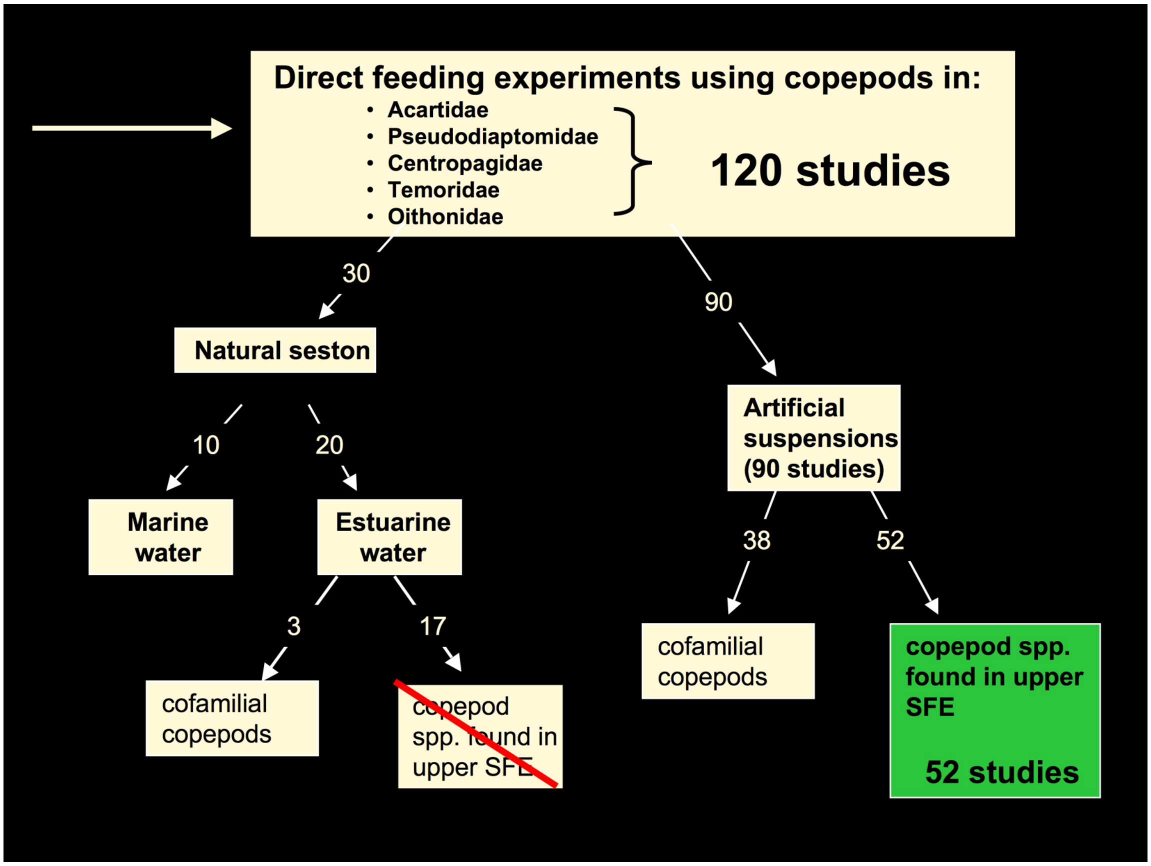
Natural Prey Taken			Heterotrophic/ Mixotrophic ciliates		Dino-flagellates	Diatoms	nano-phyto flagellates	prey <10 µm
			aloricate	loricate				
Bouley & Kimmerer (2009)	**Suisun Bay** Oct, Feb, Mar, Apr, May	**L. tetraspina**	✓	~	~		?	not counted
		P. forbesi	✓	✓	✓	✓	?	not counted
		E. affinis	✓	✓	✓	NA	?	not counted
Bollens & Penry (2003)	**San Pablo, South Bays** Feb, Mar, Apr, May	**Acartia spp.**	✓	✓	✓	✓	✓	✗

Gross Generalizations for Acartia

San Pablo Bay…. heterotrophic flagellates and ciliates > autotrophic flagellates > diatoms

South Bay………. depending on month, selectivity for aloricate ciliates, dinoflagellates, autotrophic flagellates, or diatoms

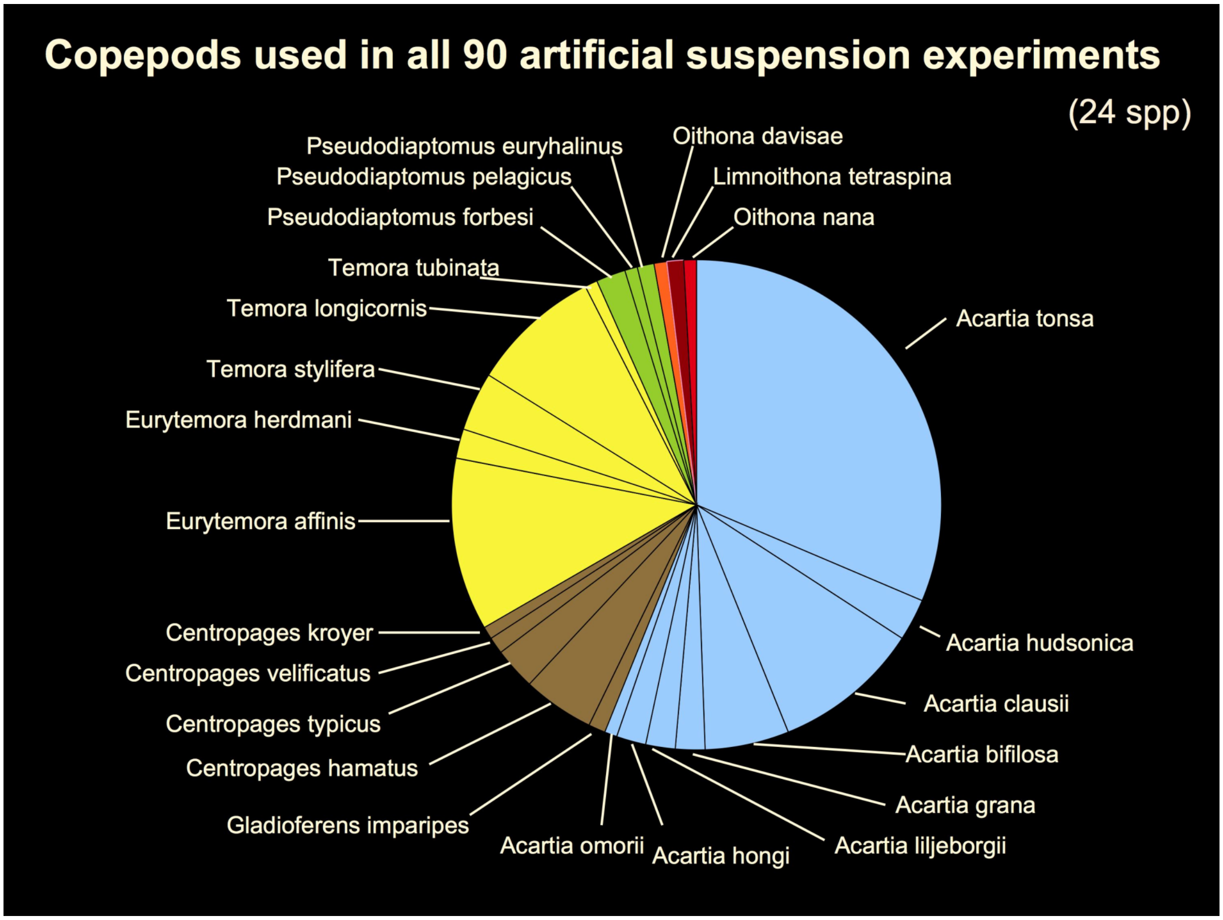
This slide grossly oversimplifies the findings from those studies.
The table indicates which categories of natural prey were taken by the principal copepod species when they were incubated in either Suisun Bay water or water from San Pablo and South Bays. With the exception of the cyclopoid Limnoithona, which appears to specialize on aloricate ciliates and other small motile prey, the *calanoid* copepods are all decidedly omnivorous and flexible in their feeding behavior.



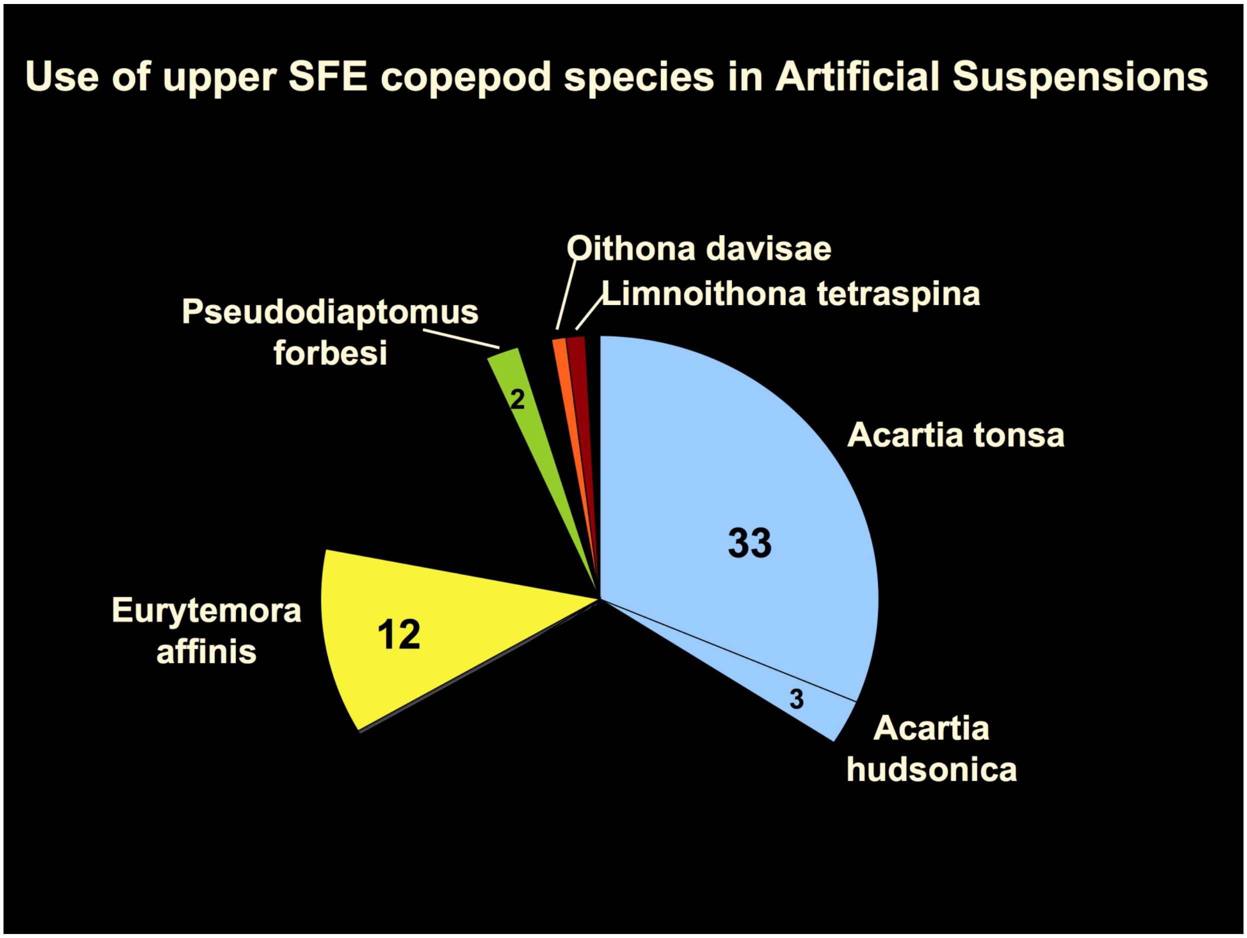
Because this information was available, I decided not to evaluate results of feeding trials using seston from *other* estuaries for this talk. Instead, I decided to focus on the 52 artificial suspension studies using copepod species found in the upper estuary, indicated by the green box. For practical reasons I sidelined the 38 artificial suspension tests using cofamilial copepods - although it would be interesting to evaluate them at a later date.

Study Designs using Artificial Suspensions

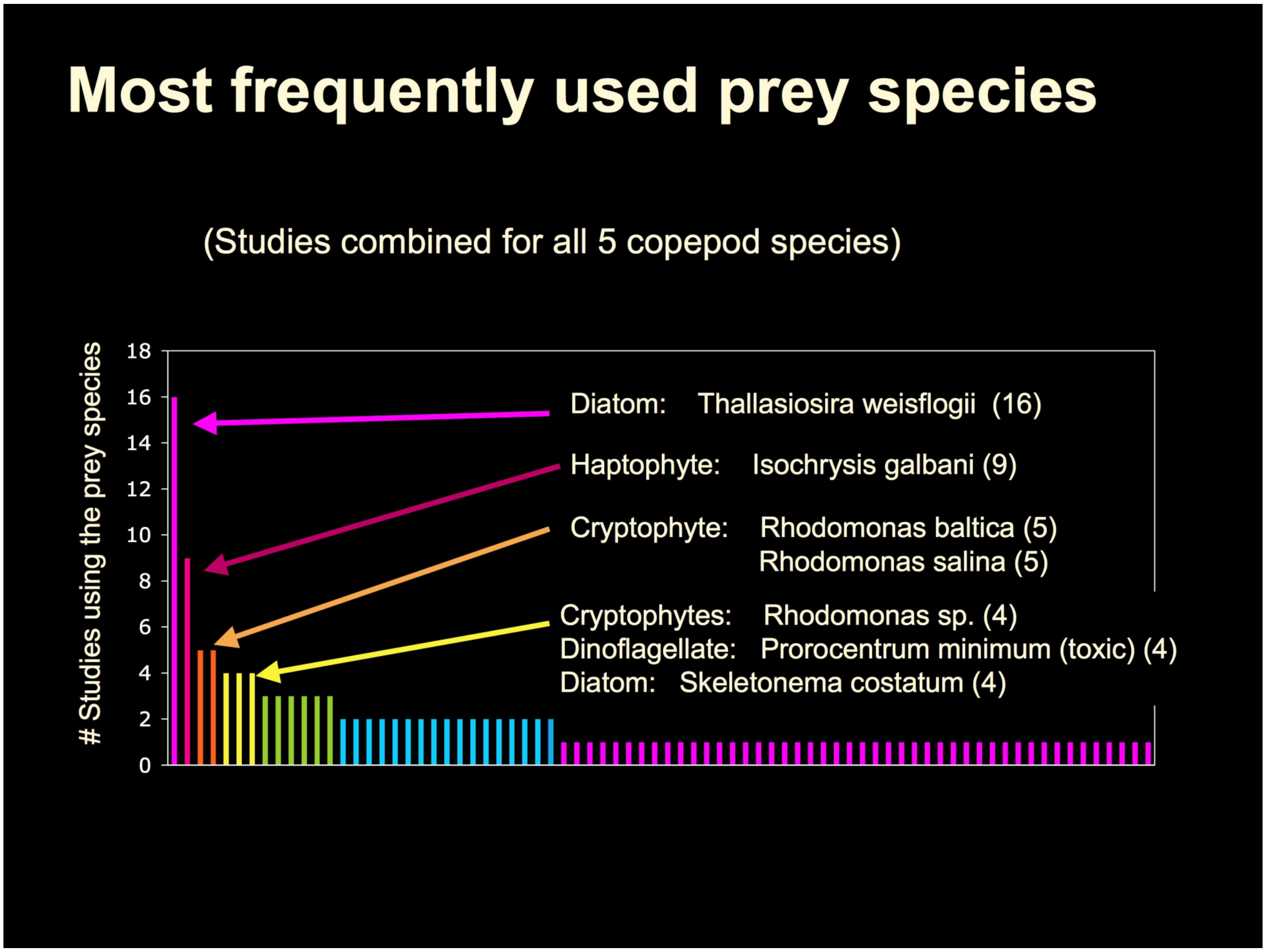
Next I'll summarize Design elements of these 52 studies



This pie chart shows that 24 copepods species were used in one or more of the 90 artificial suspension experiments - including both SFE species and their cofamilials. I know you cant read the species names -- so notice that the colors represent the five families: Members of Acartidae are blue, members of Temoridae are yellow. Very few studies involved Oithonids (which are shown in red) or the Pseudodiaptomids (shown in green). The brown represents the Centropagidae, which is represented in the estuary by the species Sinocalanus doerri.



These pie slices from the previous chart are for species found in the upper estuary. The numbers represent how many studies included the species in their experimental design.



For this slide, I ranked prey species according to their frequency of use in the experiments. The diatom Thallasiosira weisfloggi, the haptophyte Isochrysis galbani and several species of the cryptophyte Rhodomonas were most frequently used. This graph includes ciliates and dinoflagellates, but they do not score high in terms of use. This skewed distribution means we may know something about how copepods react to Thallasiosira and Rhodomonas, but our information for most prey species is almost anecdotal.

Frequency of prey offered

	Acartia tonsa	*Eury-temora affinis*	*Acartia hudson-ica*	*Pseudo-diaptomus spp.*	*Oithona davisae*	*Limn-oithona tetraspina*
Ciliates	**19**					
Diatoms	**25**	**2**	**2**	**1**	**2**	**1**
Chlorophytes	**5**	**6**		**2**		
Cryptophytes	**13**	**2**	**1**	**1**		**1**
Cyanobacteria		**3**		**2**		
Dinoflagellates	**22,12***				**1**	
Haptophytes	**15**	**4**	**2**	**3**		

*** toxic red tide dinoflagellates**

This slide tabulates the frequency of use of prey types for the 6 copepods species. Some of the interesting data gaps are shown in pink. Notice that ciliates and dinoflagellates have only really been used in feedings test with Acartia tonsa, but we know that ciliates and other motile prey are important prey items for Eurytemora and Pseudodiaptomus in the field. Also, although Limnoithona tetraspina is so abundant in the estuary, it is essentially unstudied in this context.

What was reported?		Number of exp. designs
feeding rates	prey-specific ingestion or clearance rates	39
	fecal pellet production	3
	ingestion of carbon	1
survival or reproduction	survival/ mortality	11
	secondary production (population C, #/L)	2
	egg production rate (incl. egg efficiency)	19
	hatching success	9
	development (stage reached, stage duration)	3
efficiency indices	individual growth efficiency (C or N based)	3
	population growth efficiency	2
diet chemistry	fatty acid profiles	8
	diet C:N	5
	other (proteins, amino acids, carbohydrate)	4

This slide summarizes the types of measurements that were reported in the population of studies. The parameters are grouped into 4 categories: feeding rates, survival or reproduction, efficiency indices (related to trophic efficiency) and diet chemistry.

Notice that prey-specific ingestion or clearance rates is by far the most common measurement. However, 2 indices which I consider important measures of food quality -- hatching success and development of offspring - are much more rare. In addition, I found only 8 studies in which the fatty acid profiles of prey were evaluated - despite the fact that fatty acid content is frequently invoked as a basis for gauging relative food quality.

SELECTED FINDINGS

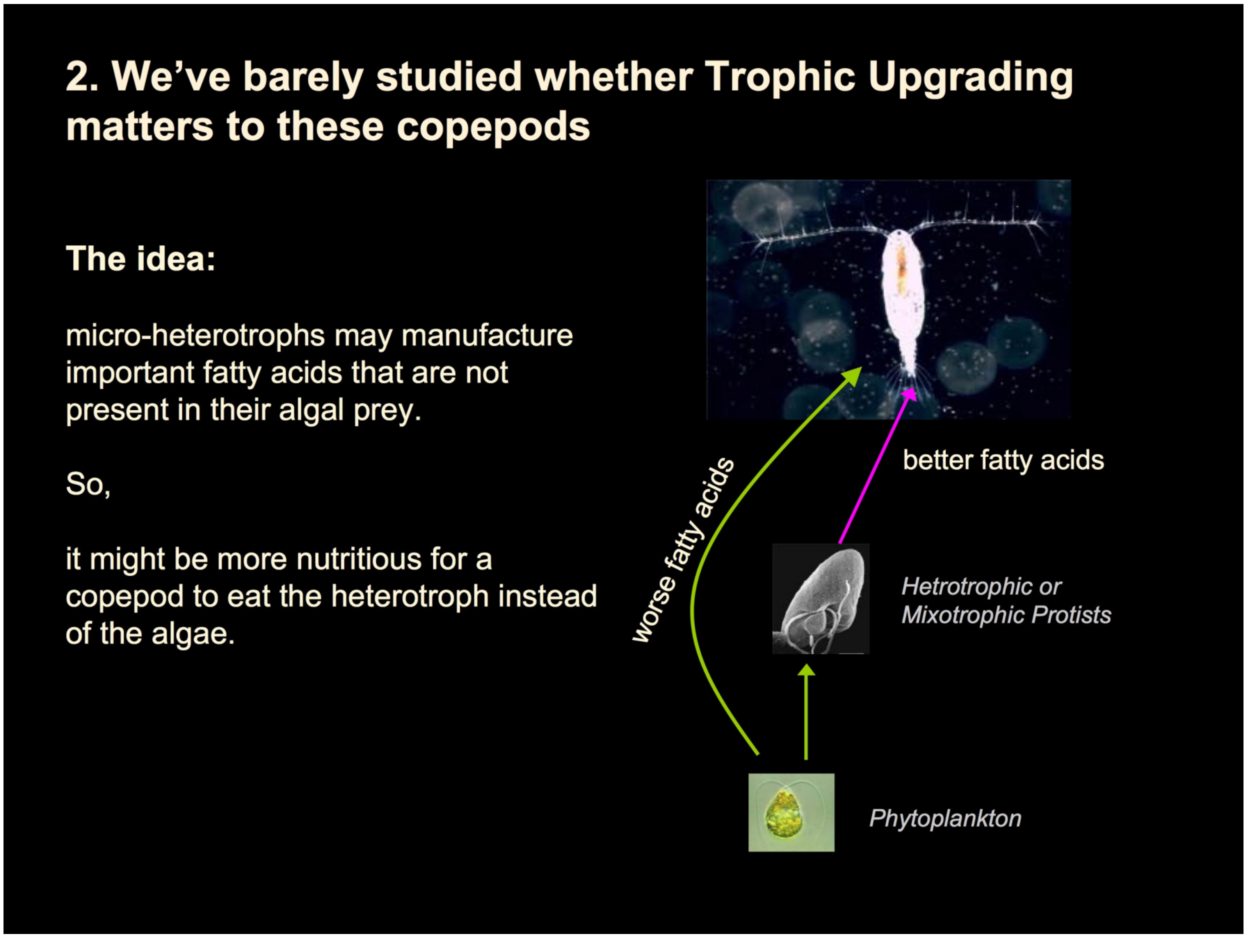
1. Fatty acid composition of diet is rarely compared to reproductive outcomes for these copepods

Fatty Acid Content determined for:	Egg Prod	Hatching Success	F1 development
Diatom	3	1	
Green			
Cryptophyte	4	1	
Haptophyte	2		
Dinoflagellate	4	1	
Ciliate	2		

only A. tonsa and A. hudsonica

1. Fatty acid composition of diet is rarely compared to reproductive outcomes for these copepods.

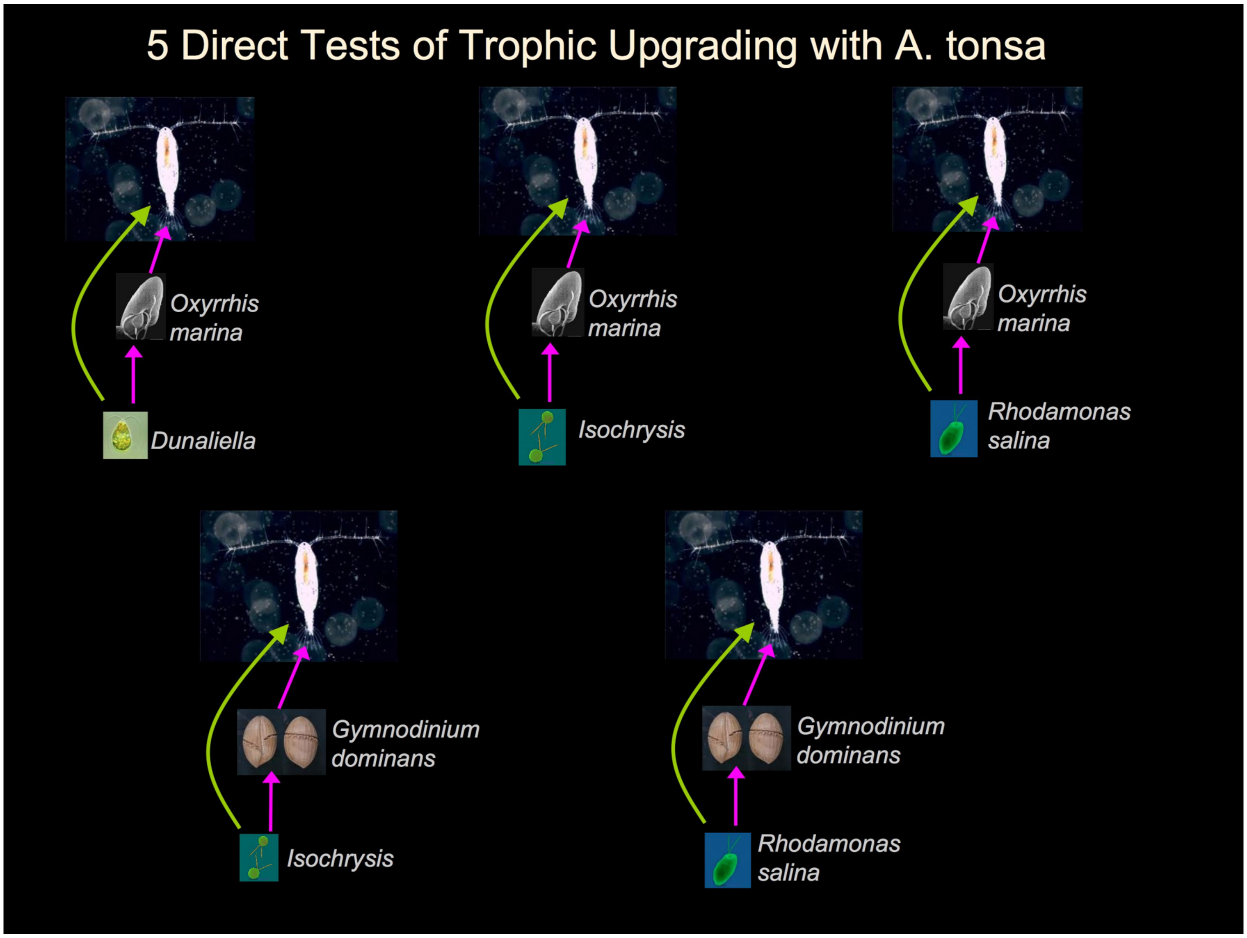
This matrix scores how many times a study measured both the fatty acid composition of prey items and some index of reproductive success. Notice that although there are a smattering of measurements of egg production in these types of studies, only one experiment measured hatching success of eggs along with fatty acid content of food offered. Note that none of the experiments measured the survival or development of offspring hatched from those eggs, in other words, F1 development. Notice that no green algae were included in these 8 tests, and that only Acartia species were used as predators.



2. We've barely studied whether Trophic Upgrading matters to these copepods

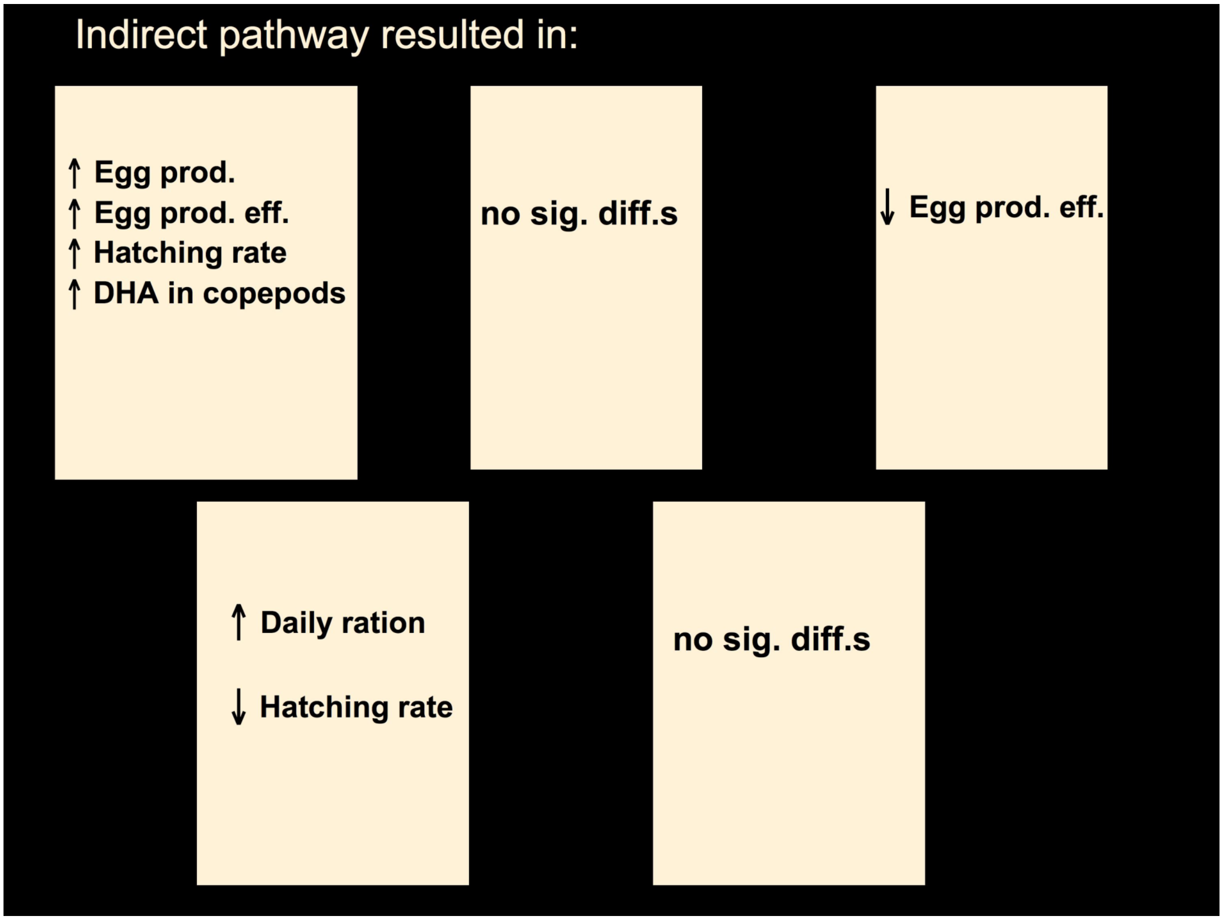
The idea behind trophic upgrading is that ciliates, dinoflagellates or other microheterotrophs might manufacture important fatty acids that are not present in their algal prey...

So, it might be more nutritious for a copepod to eat the heterotroph than the algae.

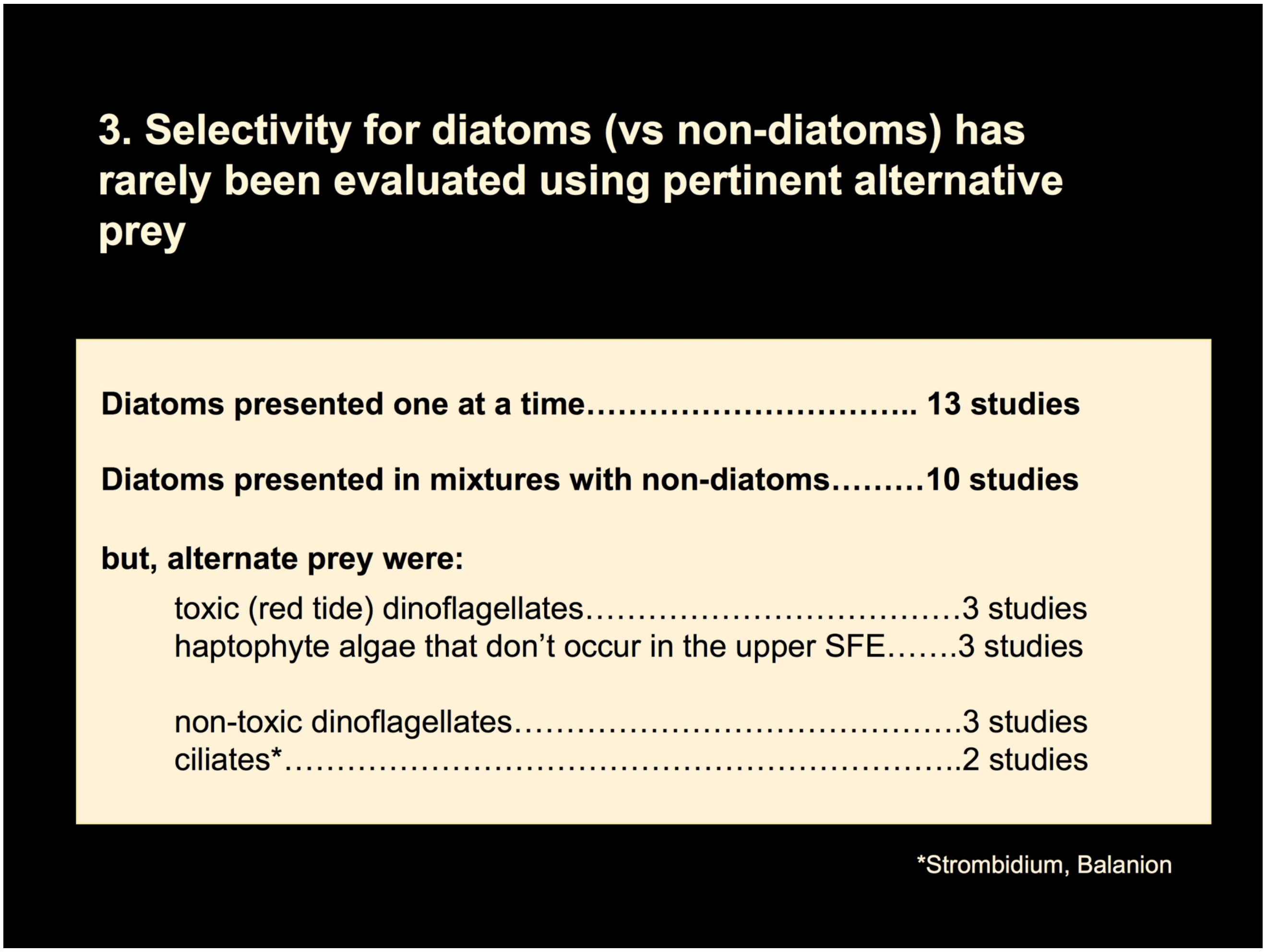


I found 5 direct tests of trophic upgrading using a SFE copepod species. This slide shows the 5 simple expermimental designs, in which the copepod was allowed to feed on either an algal species or on a small heterotroph that had been raised on the same algae.

In these tests the intermediate heterotrophs were both dinoflagellates. The algal prey were either the green flagellate Dunaliella, the haptophyte Isochrysis or the cryptophyte Rhodomonas salina.

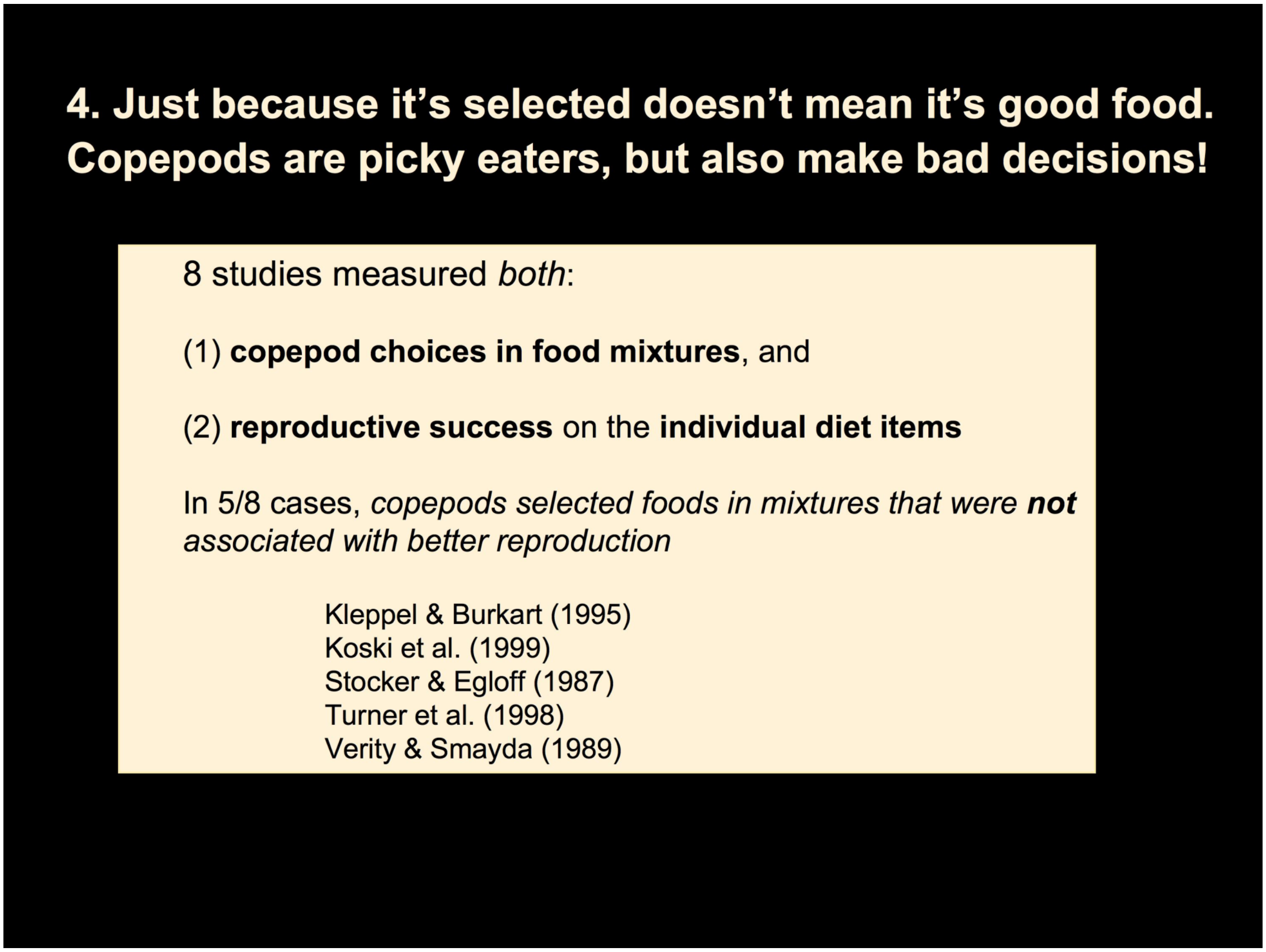


This slide shows whether performance measures were higher when the copepod ate the dinoflagellate instead of the algae directly. Notice that trophic upgrading was only observed in one of the five test designs. However, it seems pointless to generalize from such a limited data set involving so few taxa. This is clearly a data gap.



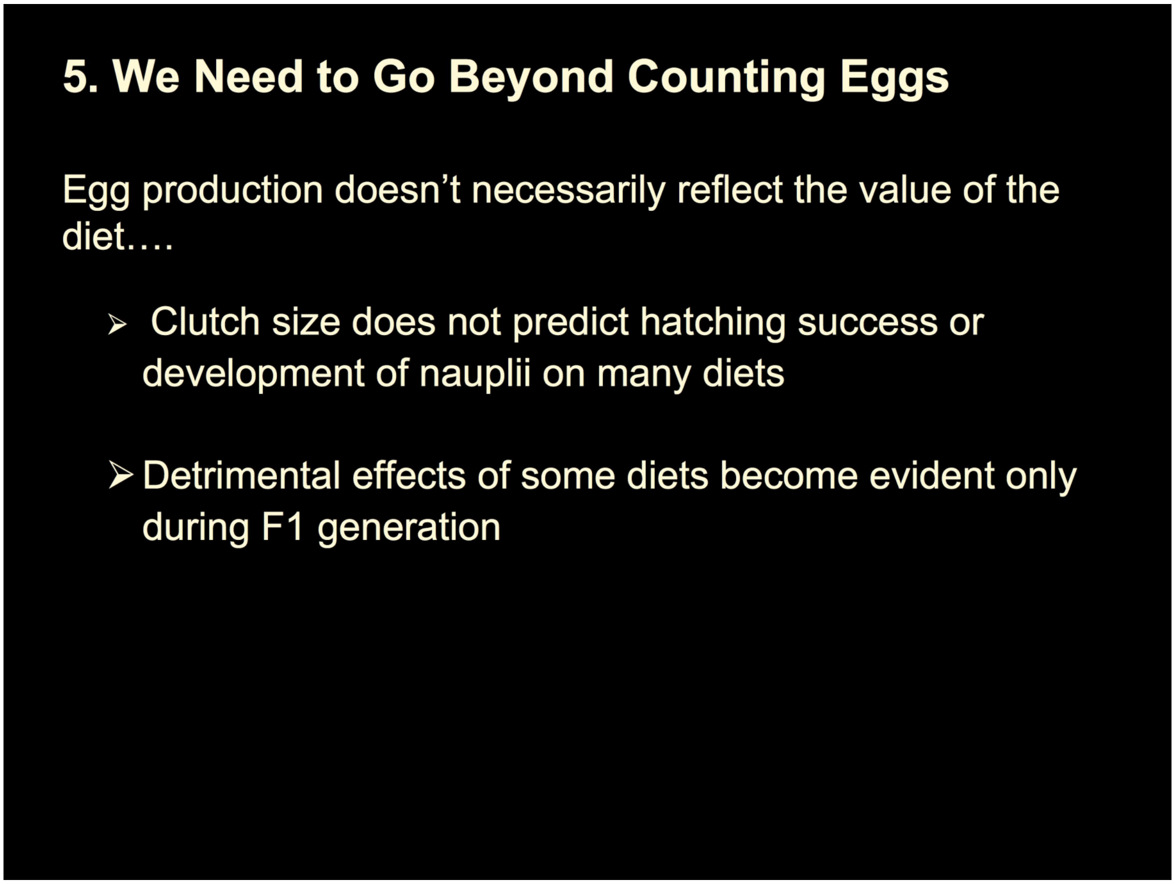
3. Selectivity for diatoms (vs non-diatoms) has rarely been evaluated using pertinent alternative prey.

I found 13 studies in which diatoms were presented one at a time, but only 10 studies in which diatoms were presented in mixtures. But in 3 of those 10 studies, the alternate prey were toxic red-tide dinoflagellates (which dont occur in the upper estuary), and in another 3 studies they were haptophyte algae that dont occur in the upper estuary. Only 2 studies used ciliates as alternate prey



4. Just because it's selected doesn't mean it's good food. Copepods are picky eaters but also make bad decisions!

I found that 8 studies measured both (1) copepod choices in food mixtures and (2) reproductive success on individual diet items. In 5/8 cases, copepods selected food in mixtures that were worse for reproductive - or showed no preference even when some foods were better for reproduction.

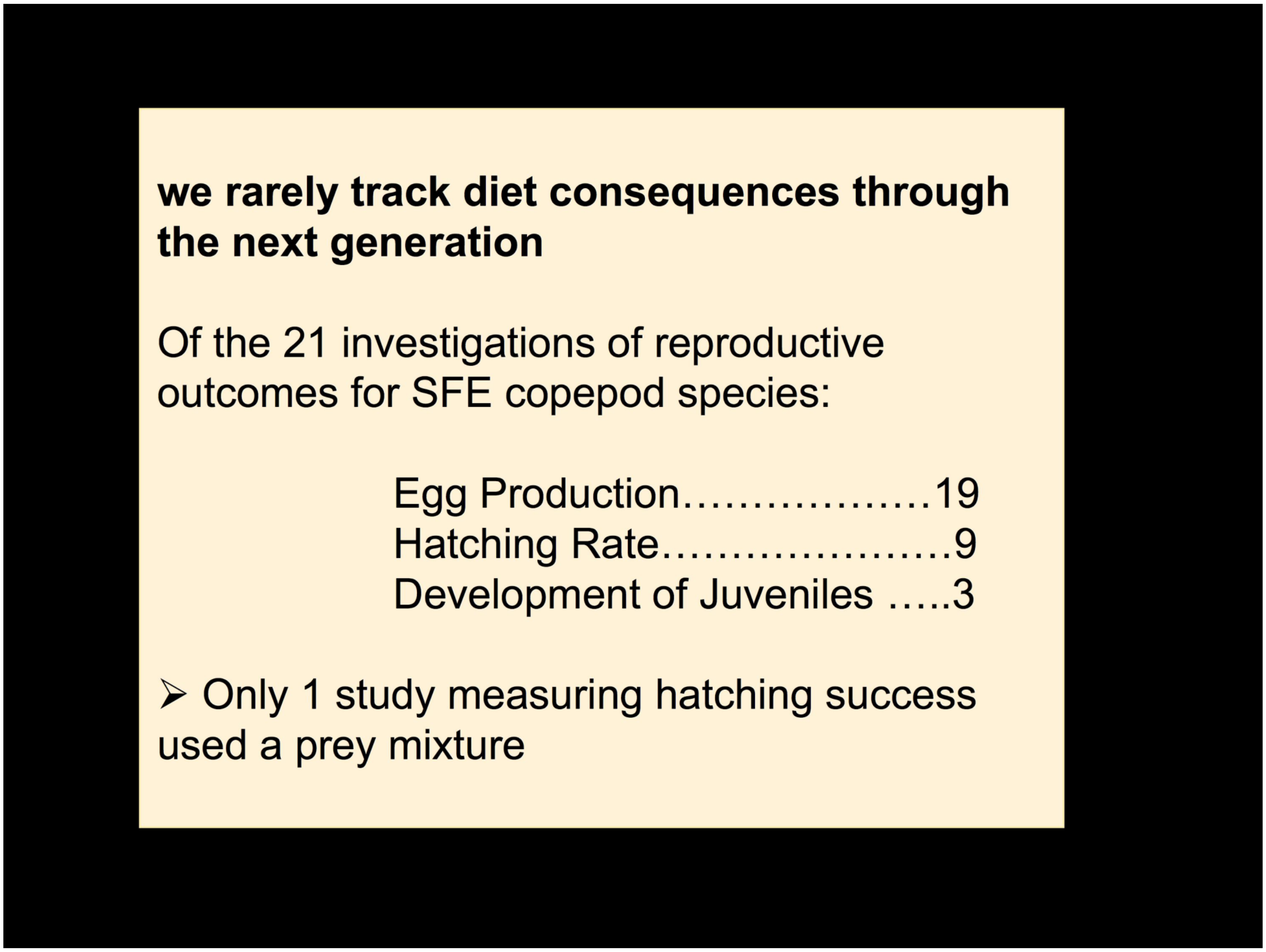


5. We Need to Go Beyond Counting Eggs

This is because egg production doesn't necessarily reflect the value of the diet because.....

Clutch size does not predict hatching success or development of nauplii for many diets

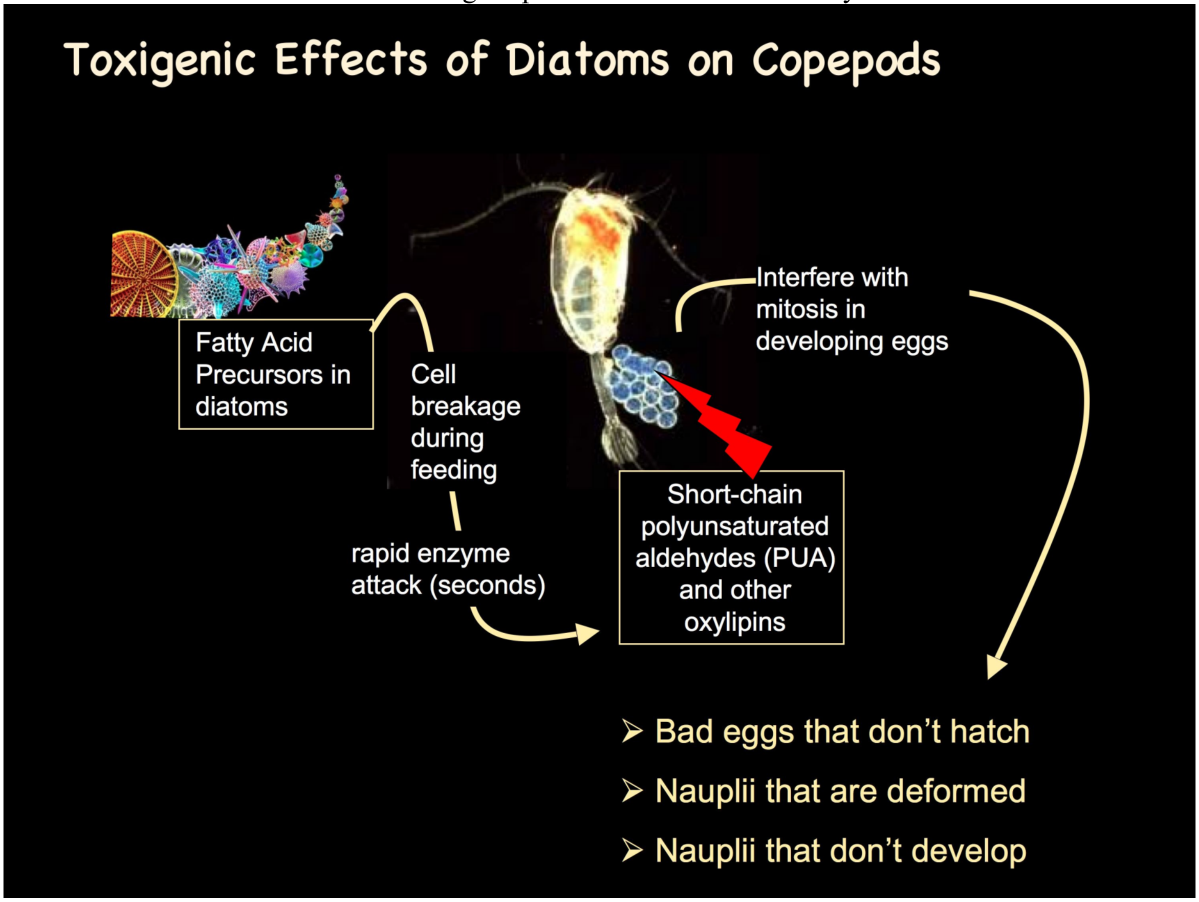
Detrimental effects of some diets become evident only during F1 generation



This slide shows that we rarely track diet consequences through the next generation

Out of the 21 investigations of reproductive outcomes of SFE copepod species, 19 measured egg production, but only 9 measured hatching rate of eggs produced while on the diet, and only 3 tracked the development of the offspring hatched from the eggs produced while on the diet.

Also, only 1 study measured hatching success for eggs produced when a copepod was fed a mixed diet.



This issue of how measure genuine reproductive success is especially important when considering potential toxigenic effects of diatoms on copepods.

This slide illustrates a phenomenon that was first observed in the lab starting in the early 1990s, but has since been studied in both lab and field settings.

The proposed mechanism is as follows:

1. Diatoms contain certan fatty acid precursors.
2. When diatom cells are broken during feeding, the precursors are released into solution and attacked by enzymes within seconds.
3. This produces short-chain polyunsatured aldehydes (PUAs) and other compounds referred to as oxylipins. These byproducts then interfere with mitosis in the developing eggs of the copepods.
4. This results in bad eggs that dont hatch, nauplii that are deformed, or nauplii that dont develop.

What's important is that the females may produce plenty of eggs while eating the diatoms -- the ill effect does not manifest until the eggs fail to hatch or the nauplii dont survive or develop properly. So merely counting eggs will not indicate whether the harmful effect has occurred. Also, raising nauplii on the diatom of interest doesn't prove anything unless the eggs were produced by females that were feeding on the diatoms. In this case, it's the maternal diet that causes the problem, not the juvenile diet.

Copepod	Diatom	Egg Prod.	Hatching Success	Normal Nauplii	Complete Develop.
Acartia tonsa	*Thalassiosira weissflogii*	−			−
	Thalassiosira pseudo nana	−			−
	Thalassiosira weissflogii	+	+		
	Chaetoceros affinis	−			
	Phaeodacylum tricornutum	−	−		
Acartia hudsonica	*Skeletonema costatum*	+			
Acartia clausi	*Thalassiosira rotula*	+	−		
Centropages typicus	*Thalassiosira rotula*	−	−		
Temora stylifera	*Thalassiosira rotula*	−		−	−
	Skeletonema costatum			−	−
	Phaeodactylum tricornutum			−	−
	Thalassiosira rotula	+	−		
	Thalassiosira weissflogii	+	−		
	Phaeodactylum tricornutum	−	−		
	Skeletonema costatum	−	−		
	Thalassiosira rotula	+	−		
Temora longicornis	*Thalassiosira rotula*				+
	Thalassiosira weissflogii				+
	Leptocylindricus danicus				+
	Skeletonema costatum				+
	Chaetoceros affinis				−
	Chaetoceros decipiens				−
	Chaetoceros socialis				−
	Thalassiosira rotula				−
	Thalassiosira pseudo nana				−
	Thalassiosira rotula	+	−		
	Thalassiosira weissflogii	+	−		
	Chaetoceros affinis	+	−		
	Leptocylindricus danicus	−	−		
	Skeletonema costatum	−	−		

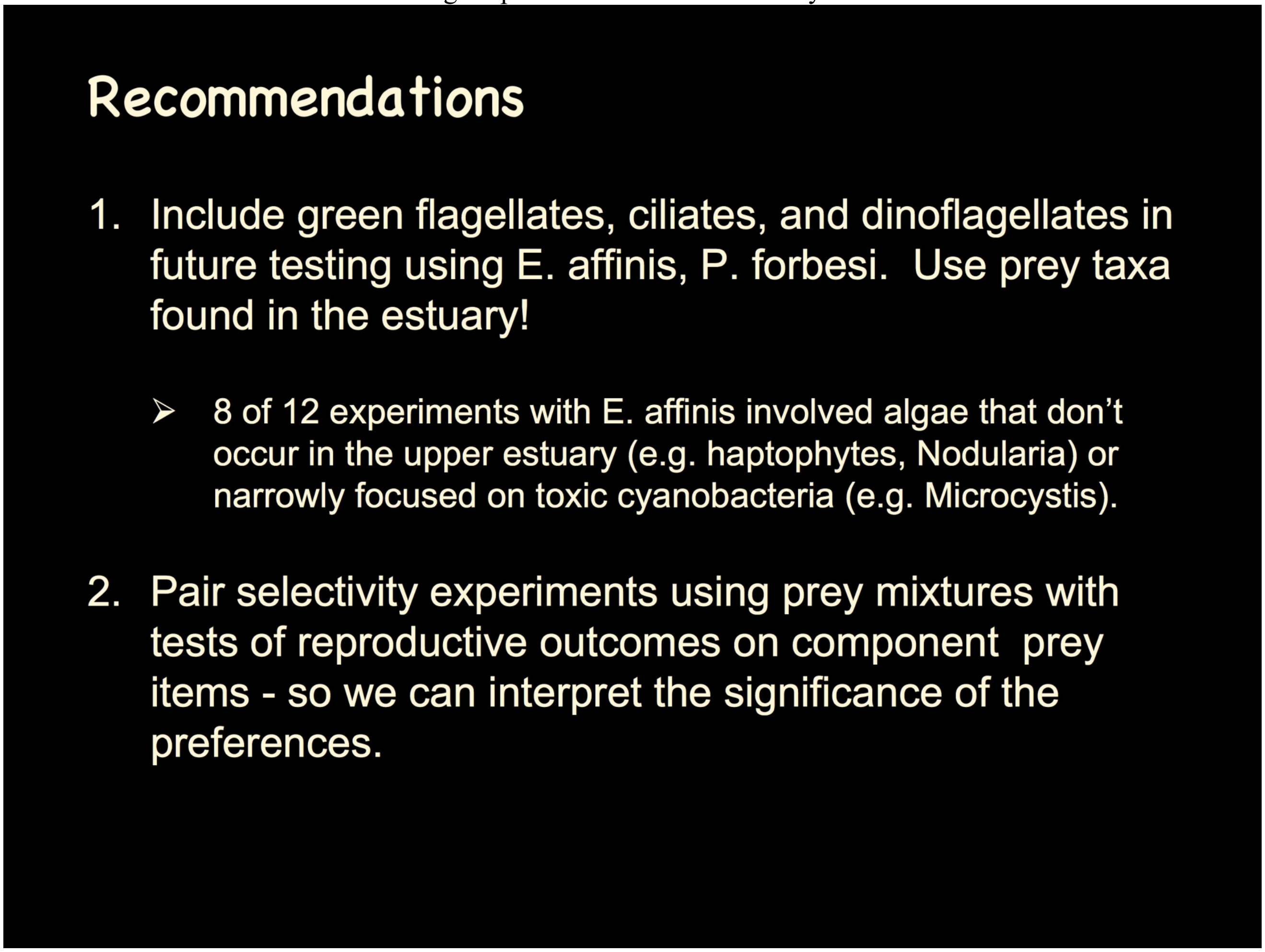
Ianora & Miralto (2010) Review

This is still a lively debate going on about this issue, which has been addressed at special symposia and in several review papers.

I was curious to see how often this phenomenon has been tested using SFE copepods species or their cofamilials.

This table is busy -- you don't need to be able to read the names of the diatom species in the second column -- each row in the table is separate test done using one copepod species and one diatom species. Scores are given for 4 types of reproductive outcomes: egg prod, hatching success, normal nauplii, and complete development of nauplii. Green indicates a successful outcome, red means failure or impairment.

Most of the entries in this table are from the recent review by Ianora & Miralto - with about 3 rows added using information from my collection of studies. As the abundance of red indicates, because many of the diatom species in the list are taxa found in the upper estuary, and because we know so little about nauplii viability on these diets, this phenomenon deserves to be included in the conversation about the pelagic food web of the San Francisco Estuary - especially given widespread assumptions that diatoms are good food for copepods in the estuary.

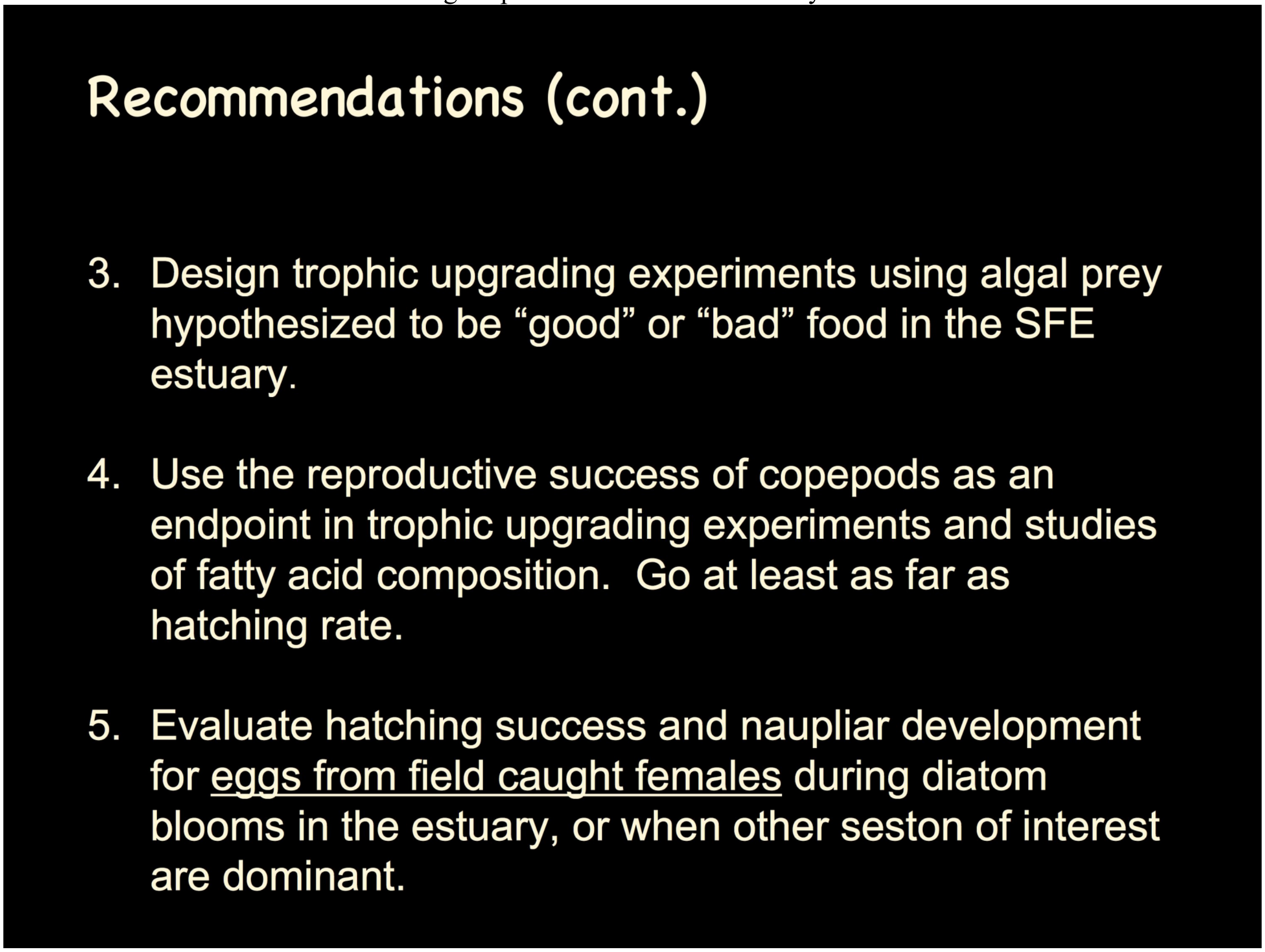


Recommendations for future studies:

1. Include green flagellates, ciliates, and dinoflagellates in future testing using E. affinis, P. forbesi. Use prey taxa found in the estuary!

I say this because 8 out of 12 of the available experiments for E. affinis involved algae that don't occur in the upper estuary (e.g. haptophytes, Nodularia) or narrowly focused on the toxic cyanobacterium Microcystis.

2. Pair selectivity experiments using mixtures with tests of reproductive outcomes on component prey items - so that we can interpret the significance of the preferences.



3. Design trophic upgrading experiments using algal prey hypothesized to be “good” and “bad” food in the SFE estuary.

4. Use the reproductive success of copepods as an endpoint in trophic upgrading experiments or studies of fatty acid composition. Go at least as far as hatching rate.

5. Evaluate hatching success and naupliar development of eggs from field caught females during diatom blooms in the estuary and other times of year when seston of interest are dominant.

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