



Widespread ciliate and dinoflagellate mixotrophy may contribute to ecosystem resilience in a subarctic sea: the northern Gulf of Alaska

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ABSTRACT: Mixotrophy among ciliates and dinoflagellates in the northern Gulf of Alaska (NGA) was widespread during spring and summer, with mixotrophs contributing a median of 38 to 61 % of total ciliate plus dinoflagellate biomass depending on season and year. The proportional contribution of mixotrophs was higher during a heatwave year (2019) than during a year of average temperatures (2018). The most common mixotrophic ciliates included *Mesodinium* spp. and several of 8 observed *Strombidium* species, while for dinoflagellates, the most common mixotrophs were *Gymnodinium*-like cells and *Tripes* (formerly *Ceratium*) spp. Onshore–offshore distribution gradients were seen mainly in summer when elevated freshwater inputs create a horizontal salinity gradient. A nearshore mixotroph assemblage consisted of nutritionally related *Mesodinium* spp. and dinoflagellate *Dinophysis*, as well as *Tripes* spp., while an offshore assemblage included *Tontonia*-like ciliates and dinoflagellates including *Gymnodinium*-like cells and *Torodinium robustum*. An 11 yr time series with lower taxonomic resolution revealed seasonality in some taxa and showed near-complete loss of *Mesodinium* spp. and *Tontonia*-like species during the longer 2014–2016 North Pacific marine heatwave. The constellation of nutritional strategies represented by NGA mixotrophs may be an important component of lower trophic level resilience to marine heatwaves, while high mixotroph contribution to ciliate plus dinoflagellate biomass may increase trophic transfer efficiency and contribute to high fisheries yields.

KEY WORDS: Protist · Mixotrophy · Seasonality · Heatwave · Microzooplankton

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1. INTRODUCTION

Mixotrophic plankton are those able to both photosynthesize and ingest prey (Flynn et al. 2019). Mixotrophy allows species to simultaneously occupy ecological niches classically considered separate: those of plants and animals. Long recognized but often considered unusual curiosities, mixotrophic protists are increasingly understood to be widespread and ecologically significant members of planktonic communities (Stoecker et al. 2009, 2017, Leles et al. 2017). Notwithstanding this recent progress, mixotrophic protist physiology, phylogenetics, and ecology remain understudied due to challenges with culturing, field observation, and experimentation; furthermore, existing knowledge is unevenly distributed among the major

mixotrophic strategies (i.e. symbiont bearing, constitutive, non-constitutive; Stoecker et al. 2017). Our focus in this study was on ciliates and dinoflagellates, although we recognize that other nano- and picoplankton-sized photosynthetic flagellates are also important mixotrophs in the northern Gulf of Alaska (NGA), as in other coastal seas (Unrein et al. 2014, Gast et al. 2018, Busse 2021, O'Hara 2023).

Mixotrophy is widespread throughout the taxonomically diverse planktonic protists, with the probable exception of the diatoms. Multiple strategies are involved. The first is true symbiosis between a feeding host and a photosynthetic endosymbiont. This strategy is found occasionally in dinoflagellates and ciliates but is widespread throughout the Rhizaria (e.g. forams, radiolarians), whose members can har-

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bor diverse photosynthetic partner species (Anderson 2014). Constitutive mixotrophs (CMs), which have innate photosynthetic capability but can also ingest prey, are widespread throughout the ocean's photosynthetic flagellates. Constituting a substantial fraction of the ocean's bacterivores (Zubkov & Tarran 2008, Unrein et al. 2014, Gast et al. 2018), larger CMs such as dinoflagellates can also ingest eukaryotic prey including ciliates and other dinoflagellates (Jeong et al. 2010). Many CMs obtain most or all of their fixed carbon through photosynthesis and ingest prey most readily when light is plentiful and dissolved nutrients are scarce, although there are numerous exceptions leading to an array of dependencies on light, dissolved nutrients, and prey concentration for the stimulation of feeding (Stoecker et al. 2017). CMs have received considerable research attention, in part because harmful bloom-forming dinoflagellates and other flagellates fall into this group (Flynn et al. 2018, Glibert & Mitra 2022).

Non-constitutive mixotrophs (NCMs) obtain their photosynthetic potential from the chloroplasts of other organisms. These are further grouped into specialists, which require chloroplasts from a limited range of prey species, versus chloroplast generalists (Mitra et al. 2016). The NCM capability is sometimes termed kleptochloroplastidy, or chloroplast stealing. Stolen chloroplasts can be retained for hours to months depending on host species and chloroplast condition (Hansen et al. 2013). Specialists *Mesodinium rubrum* and *Dinophysis* spp. occupy one end of the spectrum of both research attention and chloroplast longevity; these relatively well-studied hosts have the ability to exist photosynthetically, with no feeding or chloroplast renewal, for many weeks; they can regulate chloroplast activity (chlorophyll synthesis, protein transport, organelle division) and provide them with stress protection and inorganic nutrients from the external environment (Wisecaver & Hackett 2010, Lasek-Nesselquist et al. 2015, Tong et al. 2015, Hansen et al. 2016, Kim et al. 2016). The chloroplast retention strategies of other common NCM ciliate species, including the oligotrichs that are a major component of the ocean's microzooplankton community, are less studied and, in many cases, completely unknown, although it seems likely that there is a spectrum of strategies for acquiring and housing these organelles even among the generalists (Johnson 2011).

Models incorporating mixotrophy in an environmental context have found sometimes profound effects of the nutritional strategy on ecosystem function. Mixotrophy increased primary production in both a global and (depending on environmental con-

ditions) a regional ecosystem model (Ward & Follows 2016, Ghyyoot et al. 2017) and can affect bloom timing and resource thresholds for bloom initiation (Stickney et al. 2000, Hammer & Pitchford 2005). Mixotrophy can also be stimulatory to upper trophic level production due to increased trophic transfer efficiencies to both pelagic and benthic consumers (Ward & Follows 2016, Stoecker et al. 2017, Leles et al. 2021). These effects have clear implications for the capacity of an ecosystem to support fish, seabirds, and marine mammals from a given primary production base.

Though marine planktonic mixotrophy is receiving increasing research attention, the role of these species in the Gulf of Alaska ecosystem is unknown. This study investigated mixotrophs in the NGA (Fig. 1), a subarctic sea characterized by high environmental variability and correspondingly large gradients in the resources thought to govern mixotrophic behavior: light, nutrients, and prey concentration (Childers et al. 2005, Strom et al. 2007, 2010). The subarctic Pacific is strongly seasonal (Fig. 2), with stormy, well-mixed winter conditions yielding to a light-regulated spring diatom bloom (Henson 2007). Both warming and freshwater inputs from melting snow and ice (Beamer et al. 2016) contribute to summer stratification, associated nutrient depletion, and a community largely comprising picocyanobacteria and phytoflagellates. Coastal inputs of iron-rich freshwater also lead to strong onshore–offshore gradients in salinity, iron availability, and community structure (Strom et al. 2006), culminating in the fully iron-limited high-nutrient, low-chlorophyll waters of the oceanic Gulf of Alaska (Boyd et al. 2004).

There are several reasons to hypothesize an important role for mixotrophy in this dynamic ecosystem. First, the above-mentioned environmental drivers all vary on small spatial (<1 km) and temporal (minutes to days) scales as well as dictating the regular seasonal cycle. This creates a patchy resource environment in which availability of light, nutrients (macro- and micro-), and prey can vary manyfold over time scales comparable to protist division rates. Having multiple strategies for fixed carbon and nutrient acquisition should be advantageous in such an environment. Second, while the NGA supports only moderate annual primary production (Coyle et al. 2012), the region is home to rich fisheries for both pelagic species and, especially, groundfish and shellfish (Mundy & Hollowed 2005). Efficient trophic transfer might, in part, account for this apparent paradox. As described above, several recent models have shown that mixotrophy can increase both primary production and the efficiency with which it supports higher trophic levels.

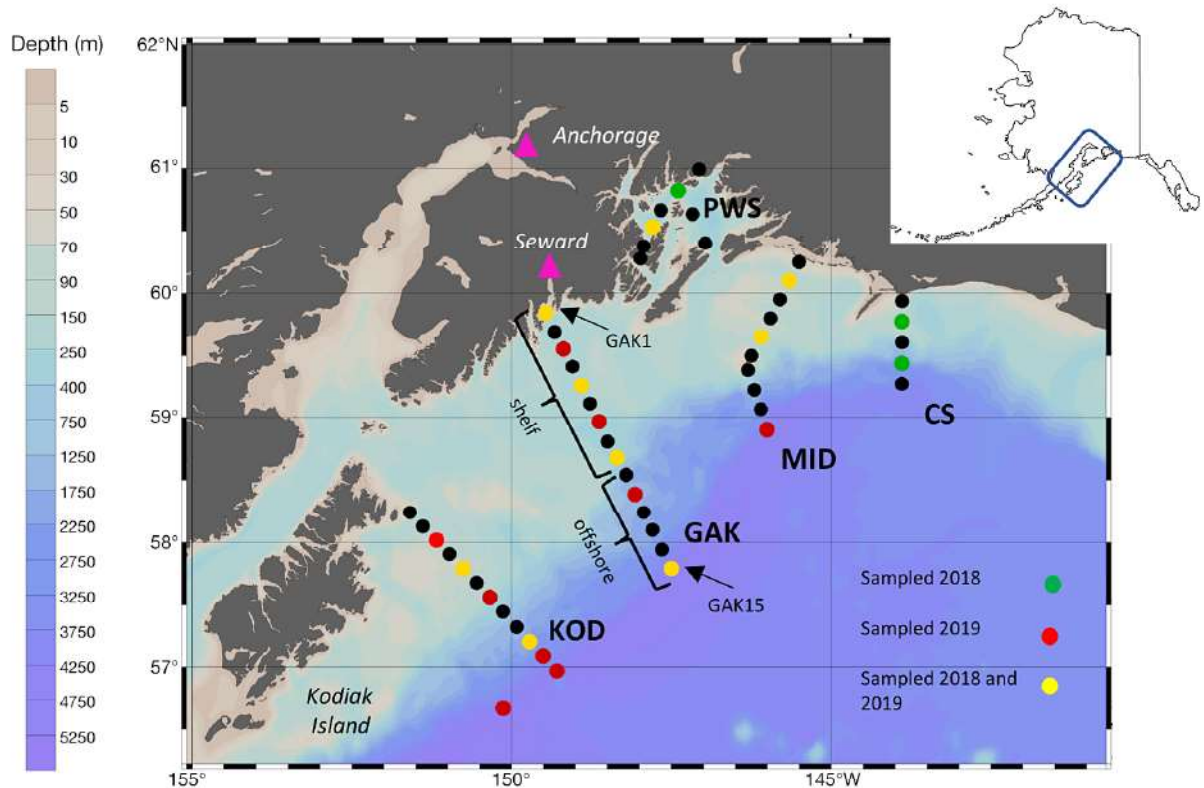


Fig. 1. Northern Gulf of Alaska study region, showing cross-shelf transect lines and stations sampled for detailed study of mixotrophic protists in 2018, 2019, or both years. Black dots show other routine sampling stations. The Seward (GAK) line extending across the shelf from Seward is the location of the 11 yr time series (locations of shelf and offshore stations are shown). Stn GAK1 is the location of the long-term temperature time series shown in Fig. 2. KOD: Kodiak; MID: Middleton; CS: Cape Suckling; PWS: Prince William Sound

An open question is the resilience of planktonic mixotrophs to large-scale disturbance. (We define resilience as the ability to recover to a pre-existing stable state of community composition and ecological function following disturbance, as in Gunderson 2000.) The NGA has recently experienced marine heatwaves that have disrupted community elements including phytoplankton size structure, krill and forage fish abundance, and seabird survival and reproduction (Suryan et al. 2021). Do the mixed nutritional strategies promoting survival in a patchy resource environment extend to survival during longer-lasting basin-scale disturbances such as marine heatwaves? Are there commonalities to the mixotrophy strategies that emerge as more (or less) successful during these events?

The NGA has recently become one of the newest sites in the National Science Foundation's Long-Term Ecological Research (LTER) network, providing an extensive framework of environmental and food web data needed to understand controls on mixotrophy. Our detailed study consisted of sampling during spring and summer of both 2018 and

2019 to characterize the abundance, composition, and range of trophic strategies comprising the ciliate and dinoflagellate mixotroph community. These 2 years provided a natural experiment in that 2018 had temperatures close to the long-term mean, while 2019 was one of the warmest years in the long-term record (Fig. 2A) with an annual temperature anomaly of $+1.2^{\circ}\text{C}$ at Stn GAK1 (Danielson et al. 2022). The NGA in general is undergoing long-term warming (Danielson et al. 2022). Given this, we also used an 11 yr (2011–2021) data set to examine longer-term spatial and temporal patterns in the occurrence of several important mixotrophic ciliate taxa as identified in the 2 yr detailed study. This longer time series encompasses a major marine heatwave that passed through the entire North Pacific, affecting the NGA from fall 2014 through 2016 (Di Lorenzo & Mantua 2016). Temperatures were the most extreme in spring 2016 (Fig. 2A), reaching 2.44°C above the mean of 6.13°C , or 3 to 4 SDs above the 20 yr average, throughout the upper 50 m of the water column.

The primary goals of our project were to (1) quantitatively analyze the mixotrophic ciliate and dinofla-

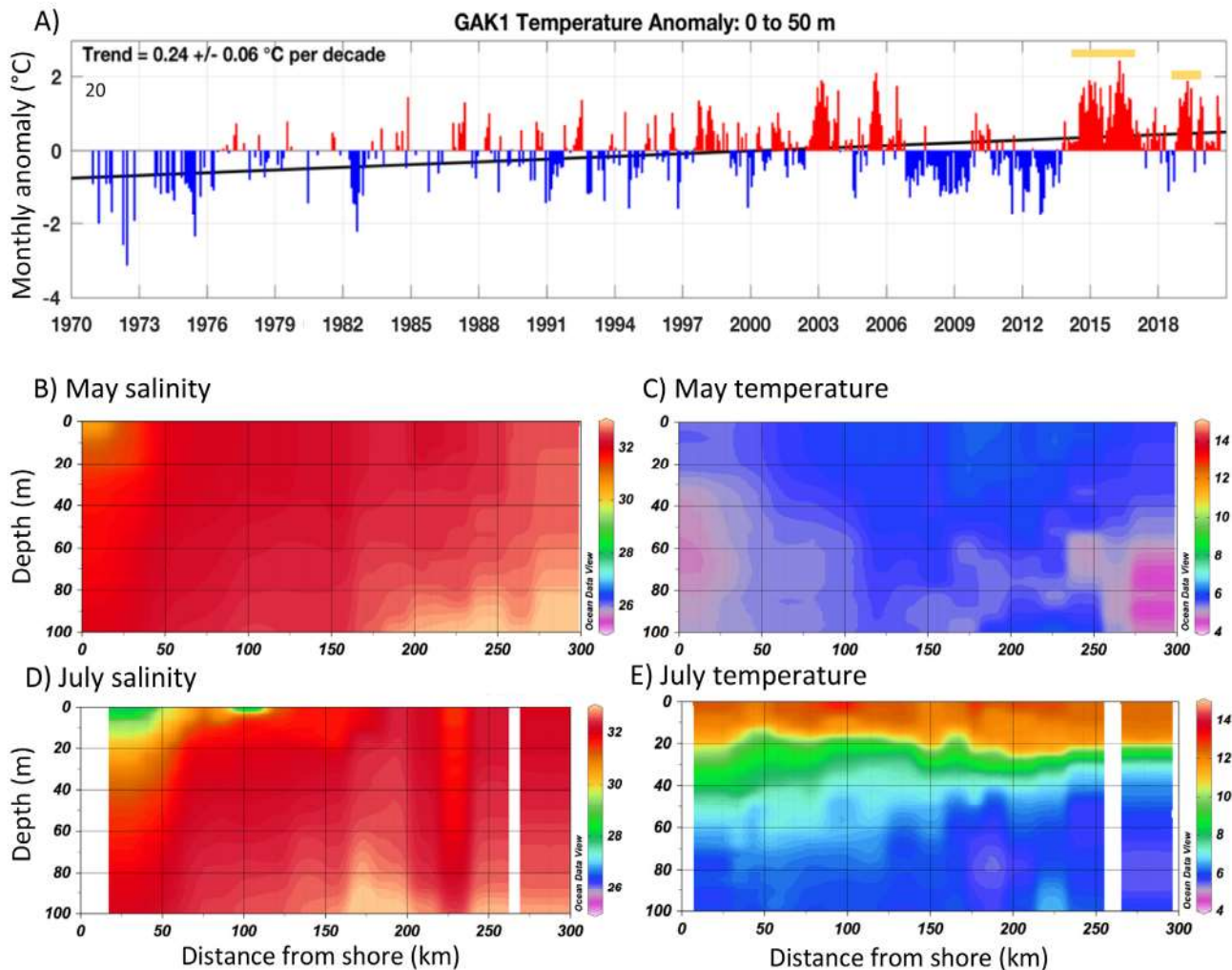


Fig. 2. Physical conditions in the northern Gulf of Alaska during the study period. (A) Fifty-year time series of temperature anomalies in the upper 50 m at Stn GAK1 on the Seward (GAK) line. Yellow bars show recent marine heatwave events, in late 2014 to 2016 and again in 2019. Water column properties in 2018, including (B,D) salinity and (C,E) temperature (in °C) as a function of depth and distance from shore along the GAK line for (B,C) early May and (D,E) mid-July

gellate communities of the NGA; (2) identify seasonal, interannual, and spatial variations in mixotroph community composition and their relationship to environmental gradients; and (3) examine responses to recent marine heatwaves to gain insights into the resilience of mixotrophic ciliates and dinoflagellates.

2. MATERIALS AND METHODS

2.1. Detailed 2 yr study of mixotrophic ciliates and dinoflagellates

Samples for the detailed analysis of ciliate and dinoflagellate community composition were collected

in the NGA in spring and summer 2018 and 2019 (Fig. 1). Sampling lines orthogonal to the coastline included (from west to east) the Kodiak (KOD) line, Seward (GAK) line, Middleton (MID) line, and Cape Suckling line. Stations in western Prince William Sound were also sampled. Samples were collected primarily from 10 m depth using Niskin bottles attached to the CTD profiler. Replicate samples were not collected.

Cells were preserved by adding seawater directly to 500 ml polypropylene bottles pre-filled with 50 ml hexamethylenetetramine-buffered 20 % (by vol.) formalin (Stoecker et al. 1989) for a final concentration of 2 % formaldehyde. Samples were kept refrigerated (4°C) at all times prior to counting and were analyzed within 3 mo of collection. For microscopy,

each sample was well mixed, and a 100 to 200 ml subsample was settled in a 250 ml conical centrifuge tube for at least 24 h. The supernatant was siphoned off, leaving a volume of <10 ml; then, the concentrated sample was mixed and transferred to a 10 ml settling chamber, and DAPI was added to stain the nuclei of the cells. This concentrated sample was settled ≥ 4 h and then examined with an inverted epifluorescent microscope to identify and measure ciliates and larger (>20 μm) dinoflagellates. Using a combination of light microscopy, blue light excitation of fluorescent light-harvesting pigments (chl *a*, phycoerythrin), and UV excitation of DAPI-stained nuclei, each cell was identified to the lowest taxonomic group possible (see below) as well as being measured and classified by trophic type. Trophic type was determined for the ciliates by evaluating any photopigment fluorescence within the cells. If photopigments were observed only in digestive vacuoles (or if no autofluorescence was present), the cell was designated a heterotroph. If photopigments were spread throughout the cell, and particularly if all autofluorescence was bright (indicating a lack of pigment degradation), the cell was designated a mixotroph. For the dinoflagellates, if there was chl *a* autofluorescence widespread in chloroplasts throughout the cell, it was designated a mixotroph, given most photosynthetic dinoflagellates are known to feed (Jeong et al. 2010). (Note that studies reporting non-feeding photosynthetic dinoflagellates remain difficult to interpret due to long-term autotrophic culture of studied dinoflagellates and lack of control for nutrient and light conditions during feeding tests.) If chl *a* autofluorescence was not seen or was present in food vacuoles only, the cell was designated a heterotroph. Dinoflagellates that had orange-fluorescing cryptophyte chloroplasts throughout were labeled as mixotrophs since these were known to be cells retaining chloroplasts from ingested *Mesodinium* spp. ciliates.

Ciliates and dinoflagellates were identified based on information in Algaebase (www.algaebase.org); World Registry of Marine Species (WORMS) (www.marinespecies.org); Planktonic Ciliate Project (www.ciliate.zooplankton.cn); and published taxonomic works including Montagnes & Taylor (1994), Steidinger & Tangen (1996), Bérard-Therriault et al. (1999), Lee et al. (2000), Horner (2002), Agatha (2004), Garcia-Cuetos et al. (2012), Gómez et al. (2016) and Johnson & Beaudoin (2019). The designation *Gymnodinium*-like was used for spherical to ovoid unarmored dinoflagellate cells with an equatorially placed cingulum exhibiting no or only slight displacement (Steidinger & Tangen 1996).

Cell biovolumes were estimated from dimensional measurements (length, width, and other measures as needed for more complex shapes) according to Hillebrand et al. (1999). Biomass of individual cells (pg C cell^{-1}) was calculated from biovolume (V , μm^3) using separate conversion algorithms for ciliates ($0.14 \text{ pg C } \mu\text{m}^{-3}$) (Putt & Stoecker 1989) and dinoflagellates ($\log \text{ pg C cell}^{-1} = \log -0.665 + (0.939 \times \log V)$) (Menden-Deuer & Lessard 2000). Data sets for this detailed study of ciliates and dinoflagellates are at DataONE (Strom & Bright 2022, doi:10.24431/rw1k6cb).

To determine which species were common, moderately common, or rare, the number of occurrences in the overall data set was totaled, and these frequencies were then ranked from most to least common. The top 25 % of taxa in terms of frequency of occurrence were considered common; these occurred at $\geq 85\%$ of stations (ciliates) and $\geq 62\%$ of stations (dinoflagellates). The bottom $\sim 50\%$ of taxa in terms of frequency of occurrence were considered rare; these taxa occurred at <30 % of stations.

Spring and summer data sets were individually analyzed using Primer 6 (version 6.1.6, Primer-E). Biomass data were square-root transformed, and non-metric multidimensional scaling (nMDS) plots were generated from the resemblance matrices based on Bray-Curtis similarity. The nMDS analysis for each season was examined using 2 factors: zone (near-shore, mid-shelf and outer shelf, offshore, eddy) and year (2018, 2019).

2.2. Ciliate and water column chlorophyll *a* content

Total and size-fractionated (>20 μm , <20 μm) water column chl *a* was determined using a cascade (serial) filtration system consisting of a 47 mm, 20 μm pore size polycarbonate filter over a 25 mm glass fiber (0.7 μm effective pore size) filter. Chl *a* content of mixotrophic ciliates was determined during the 2 cruises (spring 2018 and summer 2019) with additional *Laboea strobila* cells collected in spring 2021. Individual ciliates were picked through several rinses of filtered seawater using a drawn pipette and then transferred to small pieces of filter paper (GF/F type, 6–25 ciliates per filter). Similar volumes of ambient seawater were picked onto filter paper to serve as checks on the possible transfer of other chl *a*-containing cells (no signal was associated with these blanks). For both water column and picked ciliate samples, filters were extracted in 90% acetone and analyzed via fluoro-

metry according to previously published methods (Strom et al. 2016).

2.3. Time series of mixotrophic taxa from Lugol's samples

Samples from spring (early May) and fall (mid-September) cruises to the GAK line in the NGA have been collected since 2011, with July cruises added beginning in 2018 (data are at DataONE; Strom & Bright 2022, doi:10.24431/rwlk5ai). On each cruise, samples were collected from 10 m depth at either all GAK stations ($n = 15$) or all odd-numbered stations ($n = 8$) (Fig. 1). There are no data from 2012 or from spring 2014. Samples were preserved by addition to acid Lugol's solution for a final concentration of 5%. Details of sample processing, microscopy, and biomass estimation are described in Strom et al. (2019). Here, we include time series data from several taxa that can be identified as mixotrophs from cell morphology even in the absence of chl *a* autofluorescence (which is not visible in Lugol's-stained specimens): dinoflagellates *Tripos* spp. and *Torodinium robustum* and ciliates *Mesodinium* spp., *L. strobila*, and the *Tontonia* group (including genera *Tontonia*, *Pseudotontonia*, and *Paratontonia*). Note that some mixotrophic taxa could not be unambiguously identified in the Lugol's-preserved samples because their morphology is not sufficiently distinct from that of heterotrophic forms. These include the *Strombidium* spp. and *Gymnodinium*-like dinoflagellates. Because of these limitations, the contribution of mixotrophs to total ciliate and/or dinoflagellate biomass cannot be estimated for this time series as it was for the detailed 2018–2019 study.

Data from individual GAK stations were averaged over 2 cross-shelf zones: shelf (Stns GAK1–GAK9; 0–170 km from coast) and offshore (Stns GAK10–GAK15; 185–280 km from coast). Shelf stations have bottom depths <300 m, while offshore stations lie seaward of the shelf break with bottom depths 1500 to 4500 m (Fig. 1). The Wilcoxon signed-rank test (<https://www.socscistatistics.com/tests/signedranks/default2.aspx>) was used to test for significant differences ($\alpha = 0.05$) between shelf and offshore biomass for each of the mixotrophic taxa. A 1-tailed test was used if the detailed 2018–2019 data set had previously suggested a directional gradient; if not (*L. strobila*), a 2-tailed test was used.

3. RESULTS

3.1. Occurrence of mixotrophs during detailed 2018–2019 study

Mixotrophic ciliates and dinoflagellates were prevalent in the NGA ecosystem in both spring and summer of the 2 detailed study years (Table 1). The overall contribution of mixotrophs (median values per cruise) ranged from 38 to 61% of total ciliate plus dinoflagellate biomass. Mixotrophy was more common among the ciliates, exceeding the incidence among the dinoflagellates by up to 5 times in the warm spring of 2019. Over the entire study, mixotrophic ciliates made up 52% of total ciliate biomass (median value), while the equivalent percentage for dinoflagellates was 32%. There was a high degree of station-to-station variation in these percentages (see Fig. 3; also ranges in Table 1). Perhaps due to the highly heterogeneous nature of the NGA on these

Table 1. Median and range (in parentheses) of ciliate and > 20 μm dinoflagellate biomass in mixotroph and heterotroph categories ($\mu\text{g C l}^{-1}$) and percent of total biomass comprised of mixotrophs for stations ($n = 10$ –15) sampled on each of 4 cruises to the northern Gulf of Alaska in 2018 and 2019 and for the study overall

	2018		2019		Overall
	Spring	Summer	Spring	Summer	
Ciliates					
Mixotrophs	1.2 (0.4–4.5)	1.9 (0.2–8.0)	2.3 (0.7–11.6)	1.4 (0.2–7.4)	
Heterotrophs	1.3 (0.5–5.1)	1.7 (0.3–9.0)	2.1 (0.1–10.7)	1.0 (0.2–6.4)	
Percent mixotrophs	39 (12–83)	38 (15–90)	61 (16–97)	70 (17–94)	52 (12–97)
Dinoflagellates					
Mixotrophs	0.5 (0.1–2.2)	0.5 (0.1–5.3)	0.1 (0.0–3.2)	0.7 (0.1–3.2)	
Heterotrophs	1.6 (0.1–4.8)	1.0 (0.2–6.3)	1.1 (0.1–3.0)	0.8 (0.0–4.8)	
Percent mixotrophs	28 (5–85)	30 (3–89)	11 (0–74)	52 (29–96)	32 (0–96)
Overall					
Percent mixotrophs	38 (16–87)	38 (14–81)	44 (20–94)	61 (19–84)	46 (14–94)

sampling scales, there was no clear relationship between location and overall degree of mixotrophy for either ciliates or dinoflagellates (Fig. 3). Similarly, there was no clear seasonal difference in degree of mixotrophy, nor did we find any relationship with total water column chl *a* or the fraction of chl *a* in cells $>20\ \mu\text{m}$ (data not shown). The heatwave year of

2019 did, however, support a higher percentage of mixotrophs, with summer 2019 showing the highest biomass contribution of mixotrophs for both ciliates (70%) and dinoflagellates (52%). The somewhat lower biomass but consistently higher mixotroph contribution in 2019 is particularly evident when contrasting the summer GAK line samples (Fig. 3B,D).

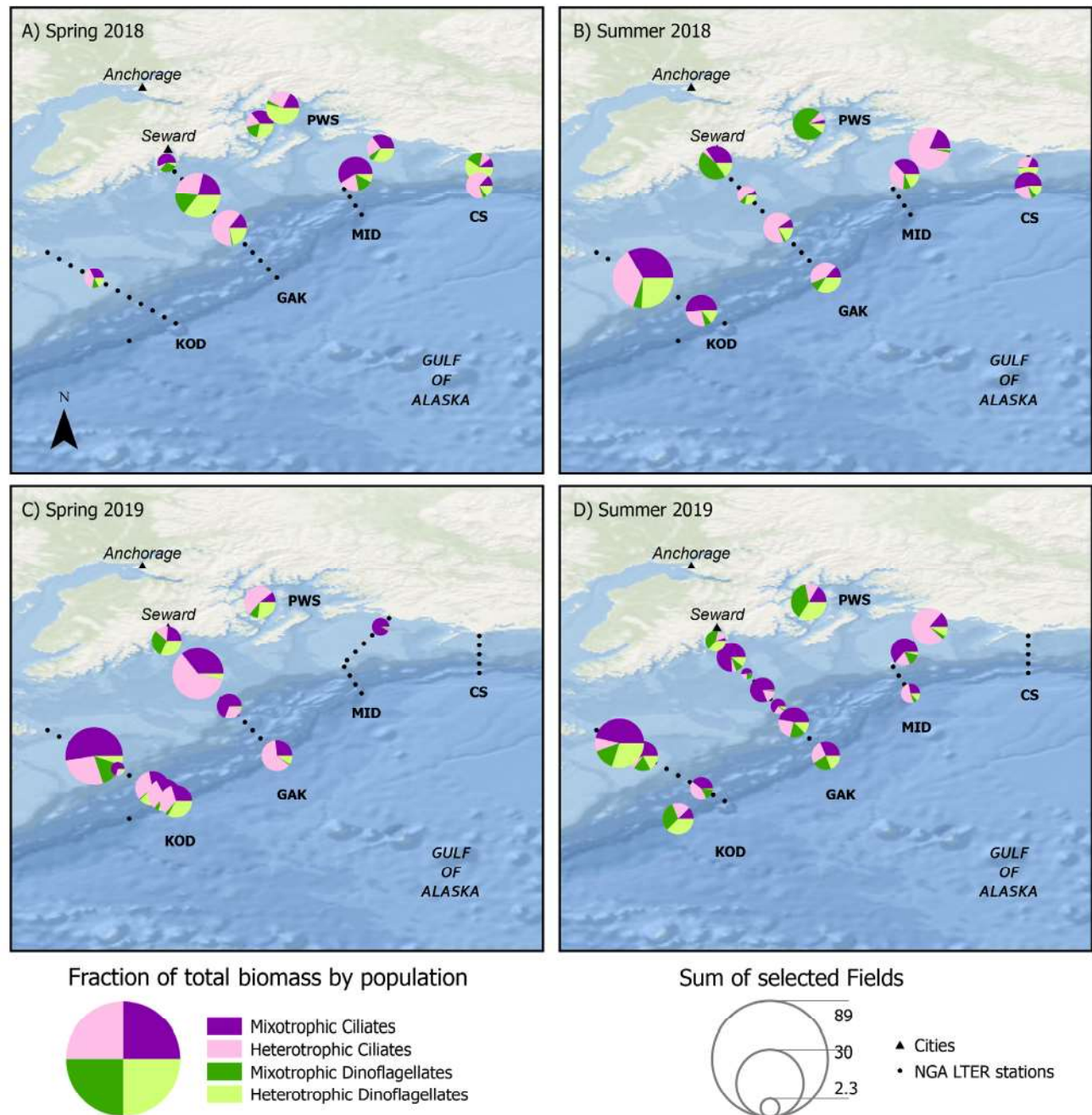


Fig. 3. Location, biomass, and composition of mixotroph plus heterotroph samples collected during spring and summer (A,B) 2018 and (C,D) 2019 in the northern Gulf of Alaska (NGA). Area of pie charts is proportional to total biomass ($\mu\text{g C l}^{-1}$) of ciliates plus $>20\ \mu\text{m}$ dinoflagellates, while division into segments shows fractional composition. Some NGA Long-Term Ecological Research (LTER) sampling stations are hidden by pie charts (see Fig. 1). Abbreviations as in Fig. 1

3.2. Composition of the mixotroph community

Epifluorescence inverted microscopy allowed us to characterize ciliate communities with a high degree of taxonomic specificity during the detailed study years of 2018 to 2019. The litostome ciliates *Mesodinium* spp. (likely a mix of *M. rubrum* and *M. major*; see Section 4.2) were the most common mixotrophic ciliates in the NGA regardless of season or year (Table 2, Fig. 4). Uniquely among ciliate taxa whether mixo- or heterotrophic, *Mesodinium* spp. were found in every sample analyzed (of 47 total samples). *Mesodinium* spp. were also a consistently important contributor to mixotrophic ciliate biomass, sometimes making up >90% of the total (e.g. Stns GAK1 and KOD5 in spring 2018). *Mesodinium* spp. presence was especially notable in the warm summer of 2019 when the genus made up >50% of mixotrophic ciliate biomass at nearly all shelf stations (Fig. 4).

After *Mesodinium* spp., the most commonly occurring mixotrophic ciliate was the large oligotrich *Laboea strobila*. This species was common during both spring and summer 2019, while it was present at only about one-third of the stations in 2018 (Fig. 4). We identified 8 species of *Strombidium* with varying degrees of occurrence (Table 2). Of these, *S. lynni* was an important contributor to biomass at several stations in spring 2018, while *S. wulffi* was a larger contributor in spring 2019 (Fig. 4). More generally, *Strombidium* spp. comprised more of the mixotrophic ciliate bio-

mass in spring than in summer (blue and green colors, Fig. 4), although the unidentified mixotrophic ciliates category in summer 2018 might have contained some *Strombidium* spp. Finally, *Tontonia*-like ciliates (genera *Tontonia*, *Pseudotontonia*, and *Paratontonia*) were moderately common throughout the study but were larger biomass contributors in summer (Fig. 4).

Although not a focus of this study, heterotrophic ciliates were also identified and their biomass contributions estimated (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a090p001_suppl.pdf). The most common heterotrophic species was *Leegaardiella ovalis*, which, like *Mesodinium* spp., was seen in nearly every sample analyzed. Also common were *Strobilidium* sp. and *Lohmaniella ovi-formis* (both especially in spring) as well as unidentified species. Tintinnids were common to moderately common and were more abundant in summer, when they made up most of the heterotrophic ciliate biomass on the MID line (Fig. S1).

Large dinoflagellates were identified in considerably less taxonomic detail than ciliates due to their lack of distinctive cell and/or nuclear morphology. Notwithstanding this coarser approach, several groups could be sorted into frequency of occurrence categories (Table 2). The most common mixotrophs were *Gymnodinium*-like cells as well as 2 *Tripos* species: *T. muelleri* (formerly *Ceratium tripos*) and *T. furca* (formerly *C. furca*). These groups were also major contributors to mixotrophic dinoflagellate biomass (Fig. 5), with *Tripos* (predominantly *T. muelleri*) especially important in summer 2019. (We also observed a third, less abundant *Tripos* species, *T. fusus* [formerly *C. fusus*].) Moderately common mixotrophic dinoflagellates included *Dinophysis* spp., *Torodinium robustum*, and unidentified thecate cells.

The most common heterotrophic dinoflagellates were also the major contributors to biomass, including *Gymnodinium*-like, *Gyrodinium*-like, and unidentified thecate cells (Fig. S2). Thecate diatom-grazing specialists *Protoperidium* spp. were also occasionally common and abundant. No marked seasonal or interannual differences were apparent in these heterotrophs, perhaps due to lack of taxonomic resolution.

3.3. Cross-shelf distribution of mixotrophic ciliates and dinoflagellates

In addition to seasonal and interannual shifts, our detailed study revealed a number of mixotrophic taxa with onshore–offshore biomass gradients. This was especially evident in summer, when high coastal fresh-

Table 2. Common, intermediate, and rare taxa of mixotrophs (ciliates and large dinoflagellates) in the northern Gulf of Alaska during spring and summer 2018 and 2019

Ciliates	Dinoflagellates
Common	
<i>Mesodinium</i> spp.	<i>Gymnodinium</i> -like
Unidentified oligotrich spp.	<i>Tripos muelleri</i>
<i>Laboea strobila</i> ^a	
Intermediate	
<i>Strombidium wulffi</i>	<i>Tripos furca</i>
<i>Strombidium epidemum</i>	Unidentified thecate spp.
<i>Strombidium lynni</i>	<i>Dinophysis</i> spp.
Unidentified <i>Strombidium</i> spp.	<i>Torodinium robustum</i>
<i>Tontonia</i> -like ^b	
Rare	
<i>Strombidium conicum</i>	<i>Tripos fusus</i>
<i>Strombidium chlorophilum</i>	<i>Erythrospidinium</i> sp.
<i>Strombidium capitatum</i>	<i>Alexandrium</i> sp.
<i>Strombidium emergens</i>	
<i>Strombidium acutum</i>	

^aCommon in 2019, rare in 2018; ^bIncludes genera *Tontonia*, *Paratontonia*, and *Pseudotontonia*

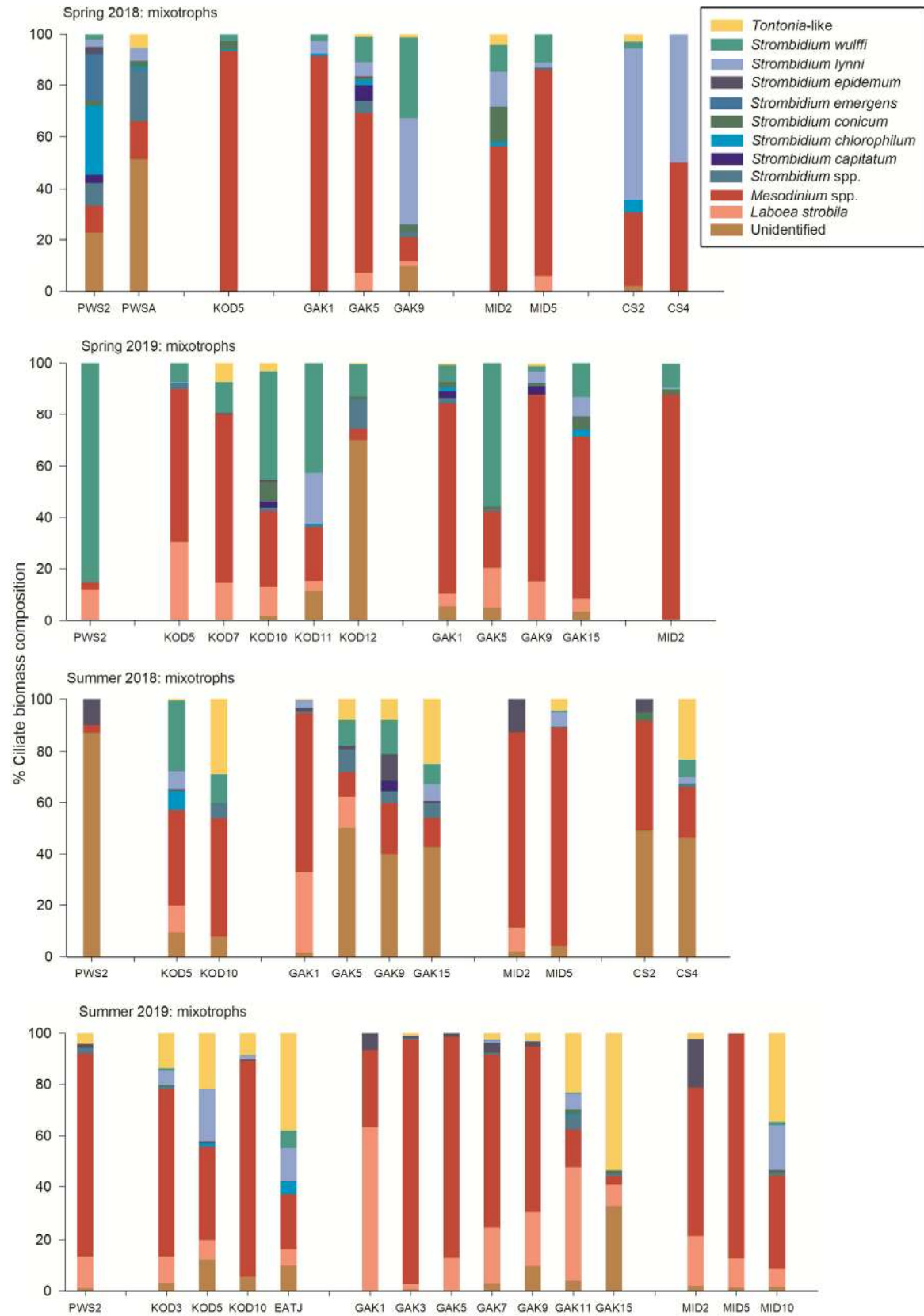


Fig. 4. Percent composition of mixotrophic ciliate communities in the northern Gulf of Alaska. *Tontonia*-like includes the genera *Tontonia*, *Pseudotontonia*, and *Paratontonia*. Abbreviations as in Fig. 1. Stn EATJ was centered in a mesoscale eddy southwest of Stn KOD10

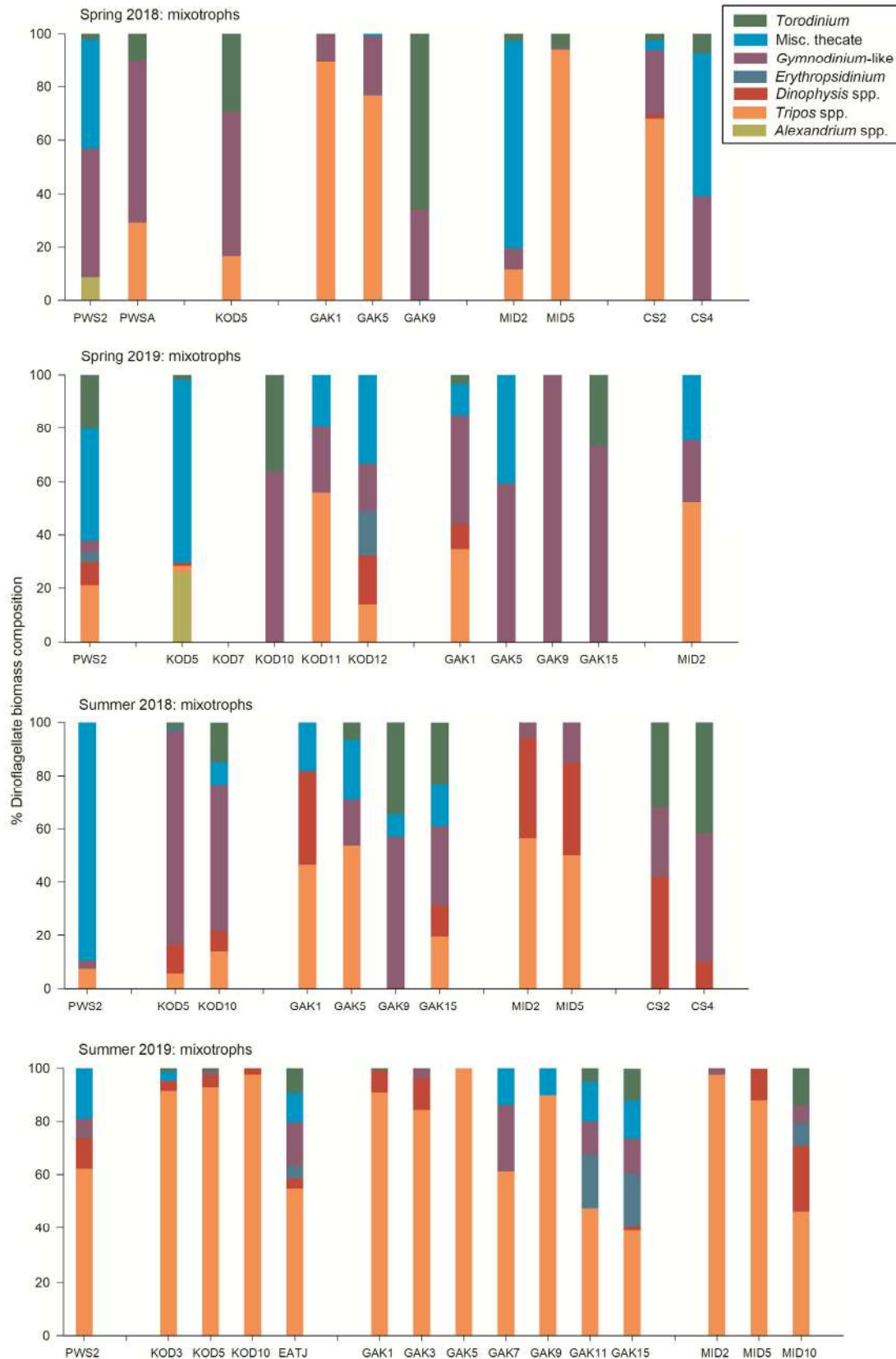


Fig. 5. Percent composition of mixotrophic dinoflagellate communities in the northern Gulf of Alaska. *Tripos* spp. includes *T. furca*, *T. muelleri*, and *T. fusus*. nd: no >20 μ m mixotrophic dinoflagellates detected in sample. Abbreviations as in Fig. 1

water runoff leads to increases in upper water column salinity as one moves offshore (Fig. 2D). Taxa increasing offshore (Fig. 6) included the large ciliate *S. lynni* (in spring), the *Tontonia* group (in summer), and the dinoflagellate *T. robustum* (in summer). Groups more abundant near shore included *Tripos* spp., *Dinophysis* sp., and *Mesodinium* spp. (Fig. 6). Both *Mesodinium* spp. and, especially, the *Gymnodinium*-like dinoflagellates showed evidence of a mixed distribution (i.e. maxima or minima at or near both ends of the transect), suggesting that these genera contain multiple species or subgroups with contrasting ecological niches.

3.4. Ciliate and water column chl *a* content

We measured the chl *a* content of 3 mixotrophic ciliate taxa while at sea in spring 2018 and summer 2019: *Strombidium* sp. (probably *S. lynni* based on comparison with preserved samples from the same location), *Tontonia*-like, and *L. strobila*. Chl *a* ranged from 42 to 92 pg cell⁻¹ (Table 3) and agreed well with the few values available from the literature for *L. strobila* and *Strombidium* spp. (Stoecker et al. 1987, 1988–1989, Putt 1990). We were unable to capture sufficient numbers of *Mesodinium* spp. to directly estimate chl *a* content, so we obtained values from 2 literature sources: a field study examining relatively small cells (Stoecker et al. 1991) and a laboratory study using larger cells raised under low versus high irradiance (Johnson & Stoecker 2005). We chose a lower middle value of 20 pg chl *a* cell⁻¹ from the range of possibilities afforded by these 2 studies, in keeping with the size range of NGA *Mesodinium* spp. and their probable irradiance environment in our 10 m samples. Based on these taxon-specific cellular chl *a* estimates, we calculated the total chl *a* content of mixotrophic ciliates in all analyzed samples and compared that value to measured total and >20 µm water column chl *a* concentrations from the same stations and depths. Mixotrophic ciliates comprised a small fraction (generally <10%) of total chl *a* in most cases (Fig. S3). The estimated ciliate contribution to chl *a* biomass and primary production in the >20 µm size fraction was higher, especially in summer, and sometimes unrealistically so (>100%; Fig. S3). These high percentages could be due to overestimation of ciliate chl *a* content and/or the fact that smaller ciliates, which can be a large proportion of the total, might slip through pores in the filters used to estimate >20 µm water column chl *a*. The data do indicate, however, that ciliates can be major contributors to >20 µm chl *a* in the summer NGA.

3.5. Insights from time series of mixotrophic taxa

The 11 yr time series from Lugol's-preserved samples corroborates some of the findings from the detailed 2018–2019 study and provides additional insights into the NGA mixotroph response to marine heatwaves. Among the taxa that could be unambiguously identified in these samples, 2 distinct responses to the longer (2014–2016) heatwave were seen (Fig. 7). Mixotrophic ciliates *Mesodinium* spp. and *Tontonia*-like, along with dinoflagellate *T. robustum*, all showed a decrease in biomass, most strongly on the shelf (green circles; Fig. 7A,C,E). *Mesodinium* spp. and *T. robustum* essentially disappeared from the shelf from fall 2014 through spring 2016, while *Tontonia*-like species showed some of the lowest biomass levels in the time series during 2016 and into spring 2017. In contrast, there was no obvious biomass decrease for ciliate *L. strobila* or dinoflagellate *Tripos* spp. in either shelf or offshore waters (Fig. 7B,D); indeed, *L. strobila* actually had higher biomass during the heatwave relative to adjacent years (Fig. 7B). The shorter 2019 heatwave did not yield the same responses. Notably, *Tripos* spp. biomass was low throughout that year (Fig. 7D), while *Mesodinium* spp. and *Tontonia*-like biomasses were unaffected (Fig. 7A,C).

Time series data also corroborate some of the onshore–offshore distribution patterns revealed by the detailed 2018–2019 study. While biomasses of *Mesodinium* spp., *Tontonia*-like species, and *Tripos* spp. were not different in shelf versus offshore waters, both *T. robustum* and *L. strobila* had significantly higher biomasses offshore (Wilcoxon signed-rank test: $z = 2.059$ and $p = 0.02$ for *T. robustum*; $z = 2.213$ and $p = 0.03$ for *L. strobila*).

Inclusion of fall data provides further insights into the seasonality of these mixotrophic taxa. In non-heatwave years, *Mesodinium* spp. were more abundant and relatively more important in spring and summer than in fall, while we saw no consistent seasonality for *L. strobila* or *Tontonia*-like cells (Fig. 7A–C). Both dinoflagellates (*Tripos* spp., *T. robustum*) exhibited biomass peaks mainly in fall (Fig. 7D,E).

4. DISCUSSION

4.1. Mixotroph prevalence in the NGA

As shown by our detailed 2018–2019 study, mixotrophs comprised about half of the ciliate plus dinoflagellate biomass during the spring and summer in the NGA (overall study median: 46%). The

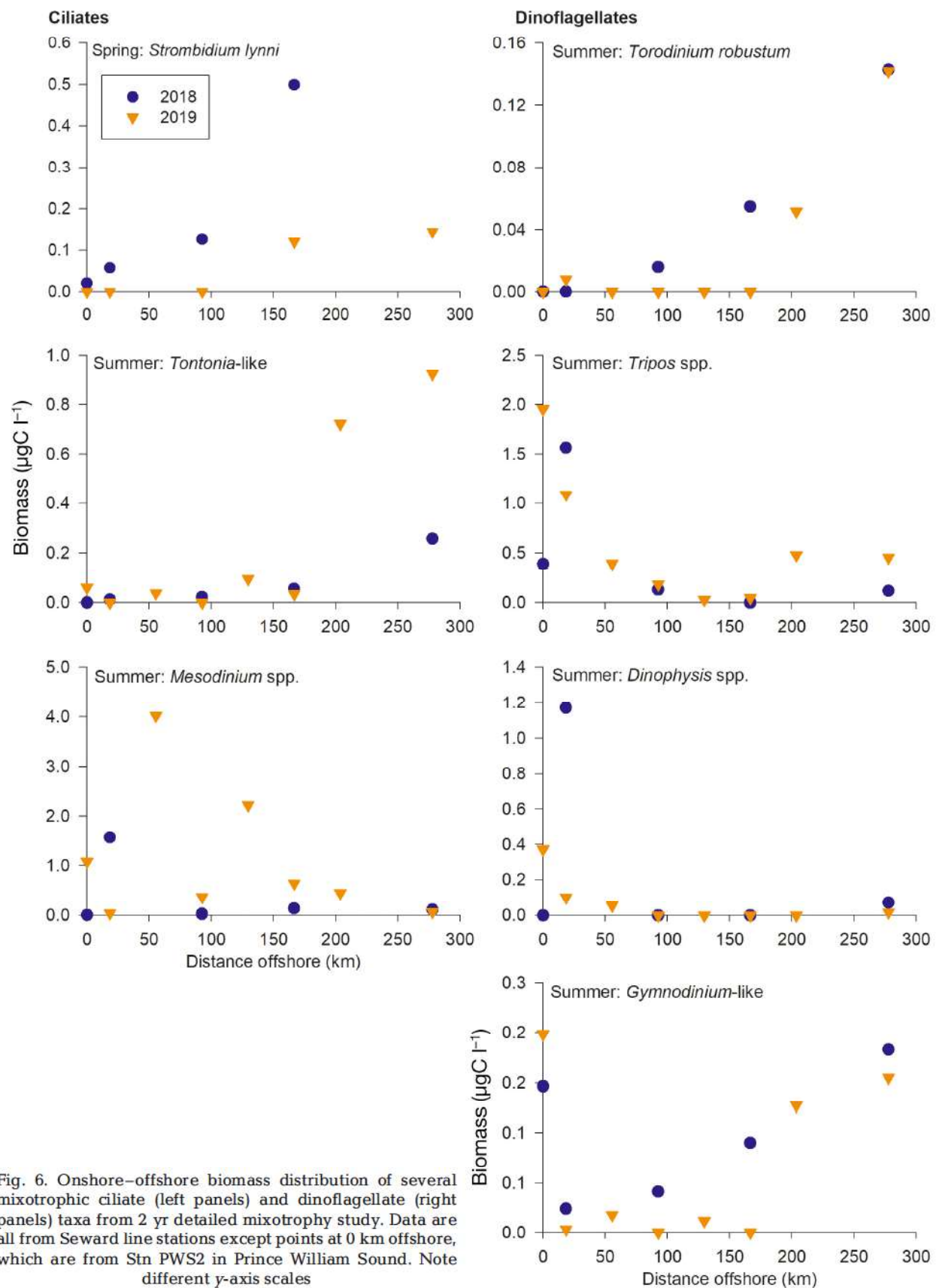


Fig. 6. Onshore-offshore biomass distribution of several mixotrophic ciliate (left panels) and dinoflagellate (right panels) taxa from 2 yr detailed mixotrophy study. Data are all from Seward line stations except points at 0 km offshore, which are from Stn PWS2 in Prince William Sound. Note different y-axis scales

Table 3. Mixotrophic ciliate chl *a* content (pg ciliate⁻¹). nd: not determined

	<i>Tontonia</i> -like	<i>Laboea strobila</i>	<i>Strombidium</i> cf. <i>wulffi</i>
Spring 2018	73	92	42
Summer 2019	50	92	82
Spring 2021	nd	87	nd

ciliate community was more mixotrophic than the dinoflagellates on every cruise (Table 1). For both groups, mixotrophs made the largest contribution to total biomass in the unusually warm summer of 2019 (70% for ciliates; 52% for dinoflagellates; 61% for both groups combined). Mixotrophic ciliates can be less prevalent in the NGA than in the Arctic (range of Arctic study averages 54–71% of ciliate biomass; reviewed by Stoecker & Lavrentyev 2018). In contrast, our findings are comparable to

the wide range reported from temperate seas (reviewed by Stoecker et al. 2009) (note that many studies do not include both oligotrich and *Mesodinium* spp. mixotrophs). Worldwide, Leles et al. (2017) found generalist NCMs (i.e. oligotrich ciliates) plus *Mesodinium* spp. to comprise 40 % of total ciliate biomass and 45 % of abundance, on average, with mixotrophy most important at high latitudes. Our data fall at the upper end of this global range, as would be hypothesized given the relatively high latitude of the NGA (58–60° N). These data support the idea that the nutritional plasticity afforded by the combination of feeding and photosynthesis is advantageous in variable high-latitude environments (Stoecker & Lavrentyev 2018). The relatively high incidence of mixotrophy in the NGA might also contribute to efficient trophic transfer, partially explaining the apparent paradox of modest annual primary production and high fisheries yields in this ecosystem (see Section 1).

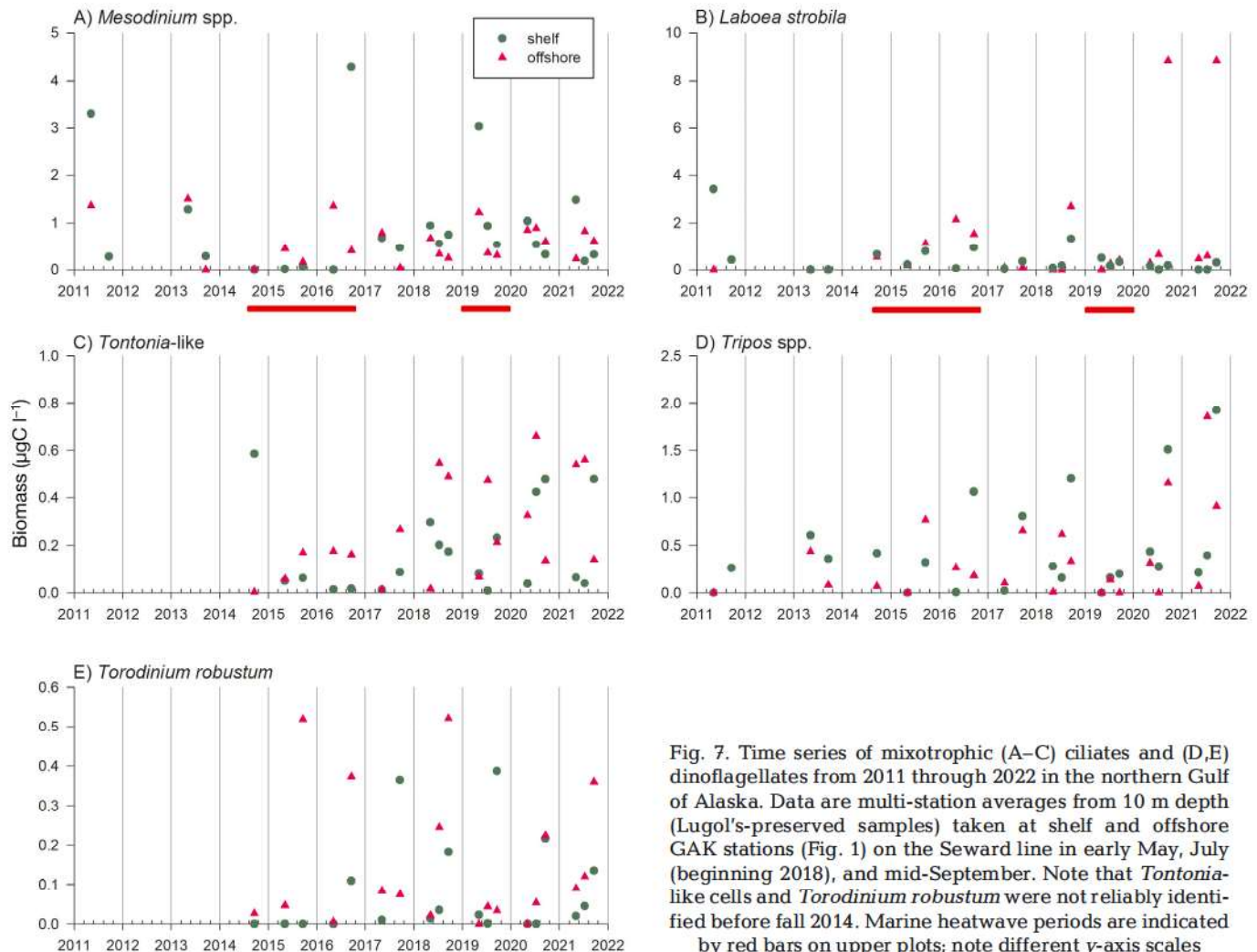


Fig. 7. Time series of mixotrophic (A–C) ciliates and (D,E) dinoflagellates from 2011 through 2022 in the northern Gulf of Alaska. Data are multi-station averages from 10 m depth (Lugol's-preserved samples) taken at shelf and offshore GAK stations (Fig. 1) on the Seward line in early May, July (beginning 2018), and mid-September. Note that *Tontonia*-like cells and *Torodinium robustum* were not reliably identified before fall 2014. Marine heatwave periods are indicated by red bars on upper plots; note different y-axis scales

In contrast to ciliates, there are few published estimates of mixotrophic dinoflagellate contribution to total dinoflagellates, especially for larger cells. For comparison with literature values, we computed ratios of autotrophic:heterotrophic dinoflagellate biomass and assumed that all autotrophic dinoflagellates are mixotrophs (see Section 2.1). Median ratios for >20 µm NGA dinoflagellates were <0.5 with the exception of the heatwave summer of 2019, when the biomass of autotrophs exceeded that of heterotrophs (Table S1). With 1 exception, autotroph:heterotroph ratios from other studies were equivalent or higher (Table S1), demonstrating that the 2018–2019 NGA dinoflagellate community was relatively heterotrophic in this broad (albeit limited) geographical context. While ratios could not be computed, a similar heterotrophic dominance was seen for most of the 2.5 yr Helgoland Roads time series in the southeastern North Sea (Löder et al. 2012).

4.2. Nutritional strategies among NGA mixotrophs

Mixotrophic ciliates and dinoflagellates encountered during this study represent a range of trophic strategies and, hence, ecological niches (Fig. 8). Among the chloroplast-retaining ciliates, the NCM *Mesodinium* spp. stood out in our study for their ubiquity and their often high contribution to total ciliate biomass; this high contribution was noted for mixotrophic *Mesodinium* spp. globally in coastal seas (Leles et al. 2017). There are 2 closely related mixotrophic *Mesodinium* species in the marine plankton, *M. rubrum* and *M. major*, both of which obligately retain cryptophyte chloroplasts (Garcia-Cuetos et al. 2012). In addition, recent molecular work indicates that *M. rubrum* may be a complex of species (Herfort et al. 2011, Johnson et al. 2016). We likely have both *M. rubrum* and *M. major* in the NGA based on the size distribution of the cells. *M. rubrum* (and likely *M. major*) possesses unique chloroplast retention strategies relative to other common NCM ciliates. In contrast to mixotrophic oligotrichs, *M. rubrum* rarely needs to feed and is capable of maintaining cryptophyte chloroplasts in an intact, photosynthetically active state for weeks to months. Thus, energy acquisition is largely from photosynthesis rather than feeding (reviewed by Stoecker et al. 2009, 2017). In addition, while *M. rubrum* can feed on a variety of phytoplankton, chloroplast acquisition is exclusively from the *Teleaulax*/*Plagioselmis*/*Geminigera* cryptophyte clade, with wild-caught *M. rubrum* nearly always containing chloroplasts from

Teleaulax amphioxeia alone (Hansen et al. 2013, Peltomaa & Johnson 2017). Finally, due to their equatorial cirri, both *M. rubrum* and *M. major* have vigorous escape jumps (Fenchel & Hansen 2006), which might enhance their success in the NGA by allowing them to escape copepod predation. Chloroplast specificity and long-term chloroplast retention appear to be successful strategies in the NGA environment, given *Mesodinium*'s ubiquity and high contribution to total ciliate biomass. However, the strategy was apparently a liability during the extended marine heatwave of 2014 to 2016 (see Section 4.4).

Little is known about the ecology or cell biology of *Tontonia* and its sister genera *Pseudotontonia* and *Paratontonia*. Our images show specificity in type of acquired chloroplast that is reminiscent of *M. rubrum* (Fig. 8K); it will be fascinating to determine the source (which is clearly non-cryptophyte) and longevity of these chloroplasts in the ciliates. In contrast, the many *Strombidium* species and the closely related *Laboea strobila* show more generalist chloroplast acquisition strategies (Fig. 8I), as also reported in a recent molecular study (Johnson & Beaudoin 2019). Laboratory studies have shown a maximum lifetime for chloroplasts in these ciliates of up to 6 d (depending on plastid type), although when ciliates are actively feeding, plastids turn over in a matter of hours (Stoecker & Silver 1990, Schoener & McManus 2012). The degree to which distribution of these ciliate species is tied to the availability of particular microalgal prey is unknown. However, in contrast to *Mesodinium* spp., *L. strobila* fared well during the extended marine heatwave, suggesting that a generalist strategy is more effective when the ecosystem is strongly disturbed for >1 yr (see Section 4.4).

Dinoflagellates arguably represent an even wider range of mixotrophy strategies than ciliates. On the mainly phototrophic end of the spectrum are the CMs: photosynthetic species that produce their own chloroplasts and appear to use ingestion, often of very small cells such as bacteria and cyanobacteria, to obtain otherwise limiting nutrients (e.g. Mitra et al. 2016). In the NGA, *Tripes* spp. represent a variation on this theme, as they are able to capture and ingest the relatively large, highly motile ciliate *Mesodinium* spp. Capture of ciliates by *Tripes* (formerly *Ceratium*) and other thecate photosynthetic dinoflagellates has been previously reported (Jacobson & Anderson 1996, Smalley & Coats 2002), although we know of only 1 previous reference to mixotrophic *Mesodinium* as a potential prey (Li et al. 1996). Other NGA mixotrophic dinoflagellates, including *Dinophysys* sp. (likely *D. acuminata*) and an unidentified

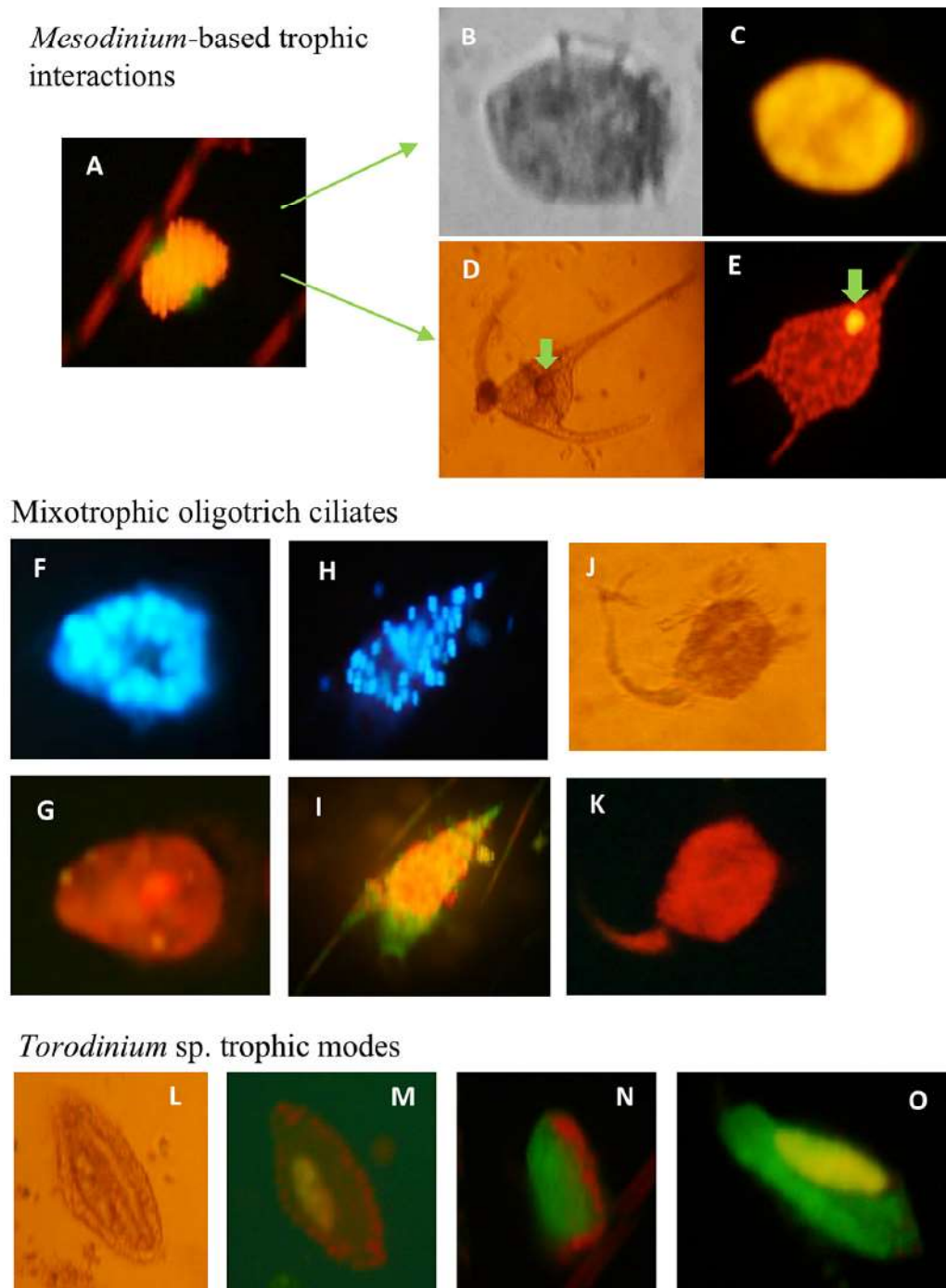


Fig. 8. Nutritional strategies among northern Gulf of Alaska mixotrophic ciliates and dinoflagellates (epifluorescence micrograph [em]; light micrograph [lm]). (A–E) *Mesodinium*-based trophic interactions. Ciliate *Mesodinium* (A, em) showing retained orange-fluorescent cryptophyte chloroplasts; dinoflagellate *Dinophysis* (B, lm; C, em), the latter showing cryptophyte chloroplasts retained from ingested *Mesodinium* ciliates; dinoflagellate *Tripos muelleri* (D, lm) and *T. furca* (E, em), the latter showing innate red-fluorescing chloroplasts, and both showing ingested *Mesodinium* ciliates (green arrows). (F–K) Mixotrophic oligotrich ciliates. *Strombidium lynni* showing multiple DAPI-stained macronuclei (F, em) and red-fluorescing retained chloroplasts (G, em); *Laboea strobila* showing multiple DAPI-stained macronuclei (H, em) and mixed orange- and red-fluorescing retained chloroplasts (I, em); *Tontonia*-like ciliate (J, lm; K, em), the latter showing uniform tiny red-fluorescing retained chloroplasts. (L–O) *Torodinium* cf. *robustum* trophic modes including lm (L), red-fluorescing, possibly innate chloroplasts plus food vacuole containing yellow-fluorescing *Synechococcus* picocyanobacteria prey (M, em), innate chloroplasts only (N, em), and *Synechococcus*-containing food vacuole only (O, em)

Dinophysis-like species that appears undescribed in the literature, adopt a *Mesodinium*-like strategy of retaining cryptophyte chloroplasts. In the case of *D. acuminata*, these are obtained indirectly through ingestion of mixotrophic *Mesodinium* ciliates (Rial et al. 2015), intimately linking the biology and ecology of these 2 mixotrophs. *Dinophysis* spp. were present but not common in our samples. Finally, *Torodinium robustum* appears to occupy the middle ground between phototrophy and phagocytosis, with a reduced chloroplast array (origin unknown) and, at times, a large food vacuole filled with the picocyanobacterial *Synechococcus* sp. (Fig. 8L–O). *T. robustum* chloroplast-like structures have been previously described (Gómez et al. 2016), but photosynthesis has not been confirmed (Boutrup et al. 2016). Published images show a vacuole corresponding to the one we saw periodically filled with *Synechococcus*-like yellow-fluorescing cells (Gómez et al. 2016), although we found no prior reports of prey ingestion. Observation of putative feeding organelles including peduncle and feeding veil has indicated mixotrophic status for the congener *T. teredo* (Gómez 2009). Little is known about these mixotrophic dinoflagellate nutritional modes in the context of natural ecosystems and their resource (irradiance, prey, nutrient) availability.

4.3. Seasonal and onshore–offshore variations in mixotrophs

Seasonality in NGA mixotrophs, as seen in both detailed 2018–2019 and longer time series data sets, did not always agree with published trends, particularly for ciliates. Globally in temperate seas, *Mesodinium* spp. are most abundant in spring, whereas generalist NCMs such as *L. strobila* and *Strombidium* spp. tend to dominate after the spring bloom during more stratified conditions (Leles et al. 2017). We did not see this in the NGA. *Mesodinium* spp. were widespread and could have high biomass, in both a relative and absolute sense, in spring, summer, and/or fall (Figs. 4 & 7A). These seasons encompass a wide range of hydrographic conditions from cold, well mixed, and nutrient rich to warm, stratified, and macronutrient deplete (Fig. 2B–E). Considering the generalists, the proportional contribution of *Strombidium* spp. was highest in spring rather than summer (Fig. 4), while *L. strobila* showed no clear seasonality (Figs. 4 & 7B). This deviation from global average seasonal patterns could have a number of underlying causes, including high species and strain diversity within some of these taxa, the availability of required

or preferred prey for chloroplast retention, and the abundance of copepod species that may regulate some of these populations through predation (e.g. Dagg et al. 2009).

The contribution of large mixotrophic dinoflagellates in the NGA tended to increase from spring into summer, particularly in the warm year of 2019 (Table 1). For *Tripos* spp. and *T. robustum*, fall was generally the time of highest abundance (Fig. 7D,E). Stratification in the NGA develops in early summer (Fig. 2D,E) and persists through sometime in September or early October depending on the timing of fall storms. Our findings suggest a successional sequence in which large dinoflagellates become increasingly established during the months following early summer stratification. The association of large mixotrophic dinoflagellates with stratified water columns has long been recognized and is thought to be related to their vertical migration ability, nutritional plasticity including mixotrophic feeding, defenses against predators, and sensitivity to shear stress associated with turbulent mixing (Margalef 1978, Smayda & Reynolds 2001, Kruk et al. 2021).

Onshore–offshore gradients in biomass were seen for both ciliate and dinoflagellate taxa in the detailed study (Fig. 6). These gradients parallel findings for mesozooplankton (Coyle & Pinchuk 2005), in which a nearshore and presumably more estuarine species assemblage transitions to an offshore, more oceanic community. Mixotroph cross-shelf gradients were mainly observed in summer, when species of *Mesodinium*, *Tripos*, and *Dinophysis* had their highest abundances in nearshore waters, while the offshore zone had peaks in *Tontonia*-like ciliates as well as dinoflagellates *T. robustum* and *Gymnodinium*-like cells. Analysis of the 11 yr time series confirmed significant offshore increases for *T. robustum* and revealed the same for *L. strobila*. (Note that sparse observations of *Dinophysis* spp. and the inability to distinguish auto- versus heterotrophic *Gymnodinium*-like cells precluded their analysis in the long-term time series.) Mixotrophic *Mesodinium* and *Dinophysis* are related through obligate trophic interactions (see Section 4.2), and both are ultimately dependent on cryptophytes, which themselves are mixotrophic and occur at higher abundances on the shelf than offshore in the NGA (O'Hara 2023). Despite these trophic relationships, we found no significant correlations between the biomass of *Mesodinium* and *Dinophysis* spp. in either the detailed or the long-term data set. Blooms of *Dinophysis* spp. are known to lag those of *M. rubrum* by up to several months (Harred & Campbell 2014, Moita et al. 2016), so our

temporally coarse sampling strategy is probably not capable of resolving these connections.

NMDS analysis of our 2 yr detailed data set included both hetero- and mixotrophic ciliates and large dinoflagellates. We found no significant stress values or groupings—including by season—when the data set was analyzed as a whole. Separate analysis of spring (May) versus summer (July) communities showed that different factors assumed greatest importance depending on season (Fig. 9). In spring, spatial gradients were of minor importance, while 2018 and 2019 communities were almost completely separated. This is consistent with the greater effects of recent heatwaves on spring than on summer phytoplankton communities (S. L. Strom et al. unpubl. data). In contrast, NMDS groupings of summer samples showed little effect of year but strong spatial separation into offshore versus shelf communities, reflective of the taxon-specific gradients described above. This cross-shelf zonation relates to the strong

seasonality in freshwater runoff into the NGA (Beamer et al. 2016), which gives rise to stronger cross-shelf salinity gradients in summer than in spring (Fig. 2) (Coyle & Pinchuk 2005).

4.4. Mixotroph response to marine heatwaves

Our 2 yr detailed study encompassed 1 marine heatwave (2019), while our 11 yr time series also included a longer, slightly more intense heatwave event (fall 2014–2016). The response of mixotrophic ciliates and dinoflagellates to these heatwaves suggests mechanisms by which the community is able to persist through such disturbances. Overall, our detailed study showed an increase in the incidence of mixotrophy during the summer 2019 event for both ciliates and dinoflagellates (we were unable to calculate the incidence of mixotrophy for the 2014–2016 event; see Section 2.3). Clearly, mixotrophy can be

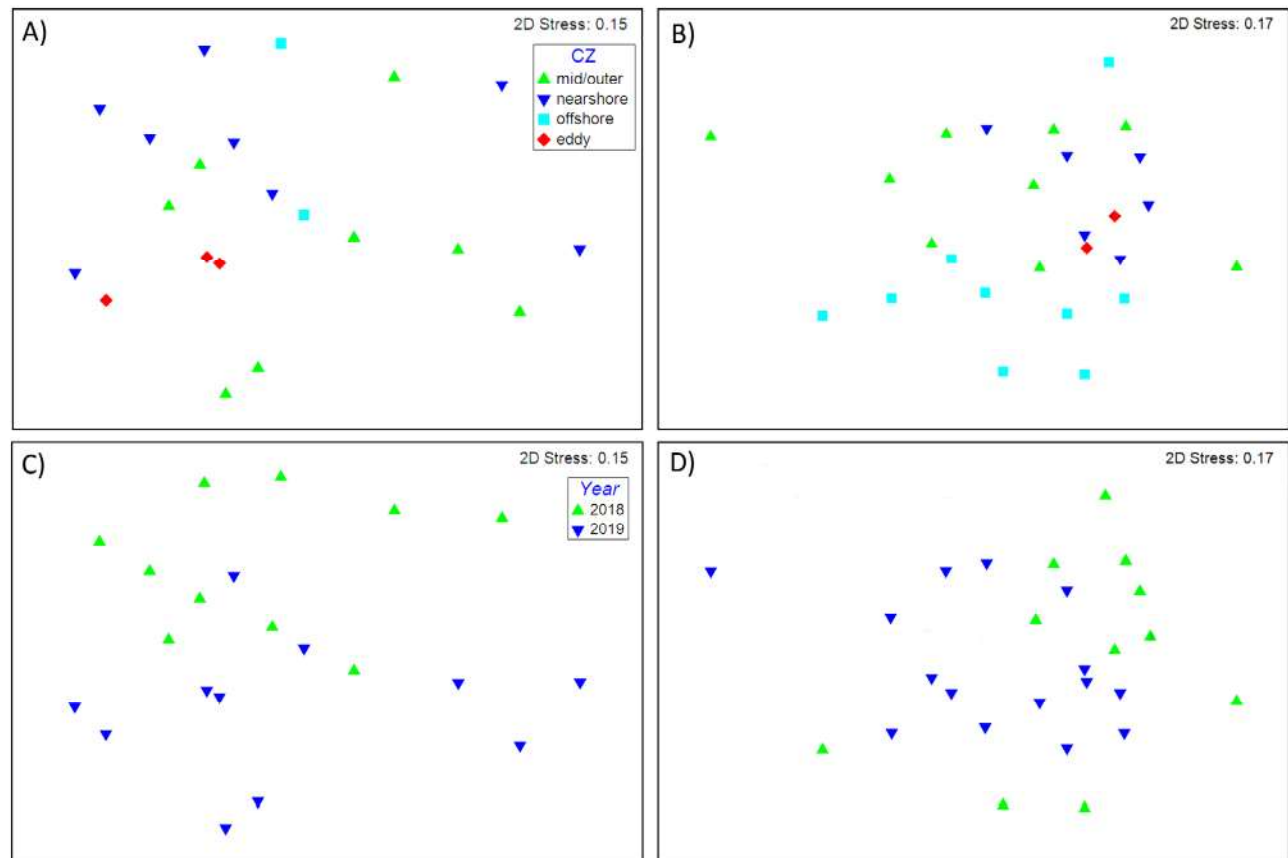


Fig. 9. Non-metric multidimensional scaling plots for detailed ciliate and dinoflagellate mixotroph data set from 2018 to 2019. (A,C) May; (B,D) July. (A,B) Data colored by coastal zone (CZ); (C,D) data colored by year. Nearshore: stations in Prince William Sound or on the shelf within 40 km of the shoreline; mid/outer: stations on the shelf between 90 and 170 km from the shoreline; offshore: stations seaward of the shelf break in waters of >1500 m depth; eddy: stations located in mesoscale eddies found on the outer end of the Seward line (summer 2018) or just seaward of the outer end of the Kodiak line (summer 2019). See Fig. 1 for location details

advantageous during warmer and more stratified conditions in this subarctic ecosystem, likely because cells can utilize multiple sources of fixed carbon (i.e. when suitable prey are scarce) and nutrients (i.e. when dissolved inorganic nutrients are low). We focus further discussion on the ciliates, for which we have higher taxonomic resolution.

The 2 main mixotrophic groups, chloroplast specialists (*Mesodinium* spp.) and chloroplast generalists (*L. strobila*; *Strombidium* spp.), showed contrasting heatwave responses. *Mesodinium* spp. were reduced to near zero during the longer heatwave but remained at high biomass (Fig. 7A) and of high relative importance (Fig. 4) during the 2019 event. *Tontonia*-like ciliates showed a nearly identical response; along with the visual uniformity of their retained chloroplasts (Fig. 8K), this suggests they, too, might be chloroplast specialists (with so far unknown chloroplast longevity). In contrast, the chloroplast generalist *L. strobila* showed no decline during the longer heatwave (Fig. 7B). *L. strobila* biomass was low during the 2019 heatwave (Fig. 7B), but relative contribution to the mixotroph community was higher than in 2018 (Fig. 4). Note that microzooplankton (ciliate plus dinoflagellate, all trophic states) biomass was reduced, relative to the long-term mean, during both heatwaves (Fig. S4).

We postulate that long heatwave duration combined with extreme temperatures creates a threshold for failure of the otherwise important NGA mixotroph *Mesodinium*. This may be tied to the availability of their required cryptophyte prey; while we have no observations from 2014 to 2016, the 2019 heatwave was associated with major decreases in cryptophyte abundance throughout the NGA (O'Hara 2023), and the same was likely true for the earlier event. Elevated temperatures per se are unlikely to be limiting; *M. rubrum* blooms are commonly observed at temperatures $\geq 5^{\circ}\text{C}$ higher than those reached during the NGA heatwave (e.g. Johnson et al. 2013, Harred & Campbell 2014). A working hypothesis is that the long chloroplast retention time (months) of *Mesodinium* spp. allows survival through a heatwave (and associated cryptophyte loss) of <1 yr duration (as in 2019) but leaves these ciliates vulnerable to a multi-year event. Resilience related to sustained environmental change might also play a role. In late 2014, the ecosystem had not seen a significant warming event for the past 7 yr, while the 2019 event was superimposed on a 5 yr period of relatively warm temperatures (Fig. 2A). Species composition and dietary and genetic adjustments within the cryptophyte–*Mesodinium* spp. assemblage in response to multi-

year warming could have contributed to the contrasting response to the 2 heatwaves.

In contrast to *Mesodinium* spp., the chloroplast generalist *L. strobila* fared well during both heatwaves (*Strombidium* spp. did as well during 2019 as in 2018 and could not be evaluated for the 2014–2016 event). Given these species' much shorter chloroplast lifetime, their success could be due to a broader range of chloroplast donor species and/or to their effectiveness at heterotrophic nutrient and energy acquisition (i.e. predation) when suitable chloroplast donors are not available (McManus et al. 2018). Overall, the range of chloroplast acquisition strategies (specialist to generalist) and the diverse assemblage (e.g. the various *Tontonia*-like and *Strombidium* spp., each likely with different feeding and chloroplast retention characteristics) likely contributed to the persistence of the ciliate mixotroph community through the 2 recent, relatively severe marine heatwaves.

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